



ESEB2025
BARCELONA 17-22 AUGUST 2025
CONGRESS OF THE EUROPEAN SOCIETY FOR
EVOLUTIONARY BIOLOGY

ABSTRACT BOOK



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EVOLUTIONARY BIOLOGY

S01 - Adaptation to environmental changes in trees through the lens of common gardens and genomics



Divergent early life-history strategies and climate sensitivity in Fir (*Abies* spp.) and Beech (*Fagus* spp.) provenances across Europe

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POSTER SESSION 1, **P01.011**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Abrupt collapse of forest ecosystems in response to heat and drought have been observed worldwide. One possible driver of these collapses is the lack of regeneration and low survival of trees in early life stages. While most research focuses on adult trees and production traits, early life stages offer insight into traits under strong environmental and potentially genetic filters.

In this study, we investigated early life-history traits including germination dynamics and phenology in fir (*Abies* spp.) and beech (*Fagus* spp.), a drought resilient and a drought sensitive species. We focused on two climate change-related stressors: shorter, warmer winters that reduce seed chilling, and warmer, drier springs that increase evaporative stress during seedling development. Using climate chamber experiments, we tested the effects of provenance and its climate and soil, seed family, and stratification (long vs. short) and temperature treatments (cool vs. warm/dry) on 34,147 seeds from 32 wild-collected provenances, averaging 10 families per provenance. Seedlings were observed every ~2.5 days over three months, covering development equivalent to the first natural growing season.

Short stratification delayed germination in both species, while heat accelerated it. *Fagus* showed reduced germination rates (i.e., the proportion of seeds that emerged with a shoot) under warm winter conditions, *Abies* maintained high germination rates despite reduced chilling and dryer and warmer springs. However, delayed

consequences of reduced stratification emerged later in development: in both genera, seedlings from some provenances exhibited halted growth under warming, revealing gene-environment interactions in establishment rates. *Abies* development was more influenced by provenance, suggesting stronger adaptive divergence, while *Fagus* showed more consistent growth across provenances, indicative of a greater colonization potential.

Provenance elevation and longitude were strongly associated with developmental responses of both genera, and adaptive trait variation was linked to soil conditions in *Fagus* and to precipitation and temperature extremes in *Abies*. These patterns point to local adaptation and species-specific vulnerabilities in the face of climate change.

Our findings show that early seedling development is shaped by both genetic background and environmental conditions, including winter chilling, spring water and heat accumulation. Predictive provenancing could help maintaining fir populations that rely on natural regeneration even under shorter winters and warmer/drier springs and summers expected in the future. In contrast, natural regeneration in beech could be further reduced under future climate change scenarios; a finding that is largely confirmed by recent research on adult beech trees. Our findings suggest that the introduction of foreign provenances would not have a better regeneration capacity either.



Estimating the demographic history of fir species (*Abies* spp.) and predicting their spatial patterns of genetic diversity

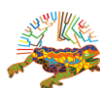
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POSTER SESSION 1, **P01.004**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Past climatic fluctuations triggered large-scale range shifts of forest tree populations and created selection pressures that favored certain traits, such as increased tolerance to heat or cold. Indeed, several common garden studies of forest trees reported that seedlings from southern and drier provenances have higher drought tolerance than northern populations. Understanding the impact of past climatic changes is essential to predict the future evolutionary response of forest trees to ongoing and predicted climate change. Fossil pollen and genomic data are our richest sources of information about past population movements of forest trees. However, inherent differences between these two data types have impeded joint analysis. Indeed, fossil records have long been considered sufficient for "storytelling" but not quantitative analysis. Here, we develop a workflow for the spatio-temporal interpolation of pollen records and propose the first joint analysis of fossil pollen records and genetic data to infer past population size fluctuations and range changes and to understand their role in determining the contemporary distribution of genetic diversity in fir species (*Abies* spp.) across Europe and Asia Minor. We developed a statistical workflow based on a unique combination of Indicator and Regression

Kriging to infer the past population size fluctuations of fir species across a grid with a 32x32km resolution and at 500-year time-steps based on fossil pollen records from 241 sites and paleo-climate data since the Last Glacial Maximum (LGM). We used Leave One Out, 5-fold and hindcast cross-validation to assess model performance. We used the resulting time series of maps of population sizes as input for a two-dimensional stepping-stone coalescent model (gridCoal). The mean coalescence time of each grid cell provides an estimate of the spatial distribution of the neutral genetic diversity today. We compared these patterns with real data, which consisted of estimates of genetic diversity. We used two data sets; one with 56 populations from the Western Alps consisting of 300 SNPs and another with 24 populations across Europe and Asia Minor from a 10k SPET genotyping panel. Our workflow allowed us to estimate the species' long-term effective population size and migration rate following the post-LGM colonization. Further, it opens the path to detecting loci under selection using a biologically meaningful null hypothesis that accounts for the demographic history of the whole species. Finally, our workflow can also help identify potential areas as seed sources for future reforestation.



Evolutionary genomics unravels the responses and adaptation to climate change in a key alpine forest tree species

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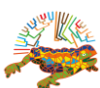
POSTER SESSION 1 - **P01.002**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Global climate change is increasingly intensifying biodiversity loss. However, it remains unclear whether the pace of evolutionary adaptation can keep up with rapid climate change across various taxa. Specifically, the relative significance and impact of genetic adaptation versus plastic responses in either facilitating or hindering climate change responses are largely unexplored. Forest trees, as foundational components, and primary carbon sinks in terrestrial ecosystems, play a crucial role in combating and mitigating the effects of climate change. Yet, their long lifespans, large body sizes, and often lengthy generation times make them particularly vulnerable to maladaptation under changing climatic conditions. Some climate-related threats may even emerge within the lifespan of individual trees. Understanding the adaptive strategies of tree species is therefore essential to inform conservation efforts and guide effective management under a changing climate.

Materials and methods: In this study, we focus on *Populus lasiocarpa*, a key dioecious tree species whose range includes the Hengduan Mountains of China, a global biodiversity hotspot known for its complex and heterogeneous environments. We constructed a de-novo chromosome-level reference genome of *P. lasiocarpa* and subsequently acquired whole-genome re-sequencing data from 200 individuals representing 22 populations across its natural distribution range. Additionally, we obtained RNA-seq data for experimental seedlings from western and eastern populations, reflecting adaptation to distinct thermal and waterlogging environments under both control and stress conditions in leaf and root tissues. With this comprehensive genomic and transcriptomic dataset, we examine the roles of long-term

natural selection, local environmental adaptation, and immediate gene expression plasticity in driving evolutionary adaptation to climate change in this species. Additionally, we integrate space-for-time approaches with machine learning to calculate the genetic offset and assess genomic vulnerability across the species' natural range.

Results and conclusion: Over historical timescales, our findings demonstrate that demographic dynamics, divergent selection, and long-term balancing selection have shaped and maintained genetic variation within and between populations. In examining genomic signatures of contemporary climate adaptation, we found that haplotype blocks, characterized by inversion polymorphisms that suppress recombination, play a crucial role in clustering environmentally adaptive variations. We further assessed the relative contributions of environmentally induced plastic responses, constitutive expression divergence between genetic clusters, and their interactions in driving gene expression variation and divergence. Notably, we observed a strong correlation between sequence divergence and constitutive differential expression among genetic clusters. Finally, by incorporating local adaptation, migration, and genetic load into our predictions of population-level climate change risks, we identified western populations—primarily distributed in the Hengduan Mountains—as the most vulnerable to climate change. These populations should be prioritized for conservation and management. Overall, our study advances understanding of the relative roles of long-term natural selection, local environmental adaptation, and immediate plasticity responses in driving evolutionary adaptation to climate change in keystone species.



FruitRescue: Large-Scale Fitness Phenotyping of Fruit Trees for Climate Resilience across multiple environments in Europe.

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POSTER SESSION 1 - P01.006, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: To provide a comprehensive assessment of the vulnerability of fruit trees and their wild relatives to climate change, a European consortium of researchers called FruitRescue was formed. Bringing together experts on several emblematic woody perennial species and their wild relatives (apple, apricot, peach, grapevine, and olive), the consortium seeks to create a system for predicting how well these species adapt to different local environments.

Material and methods

Genomic offset will be calculated using various tools to predict local adaptation. To confirm these predictions, a European network of common gardens has been established and is being monitored. Phenotypic and phenological data from this trial is shared among the partners, curated, and stored in a SQL database for real-time access.

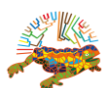
An R Shiny portal facilitates data upload via formatted CSV files, visualization through standardized plots, and data retrieval in

compliance with the MIAPPE (Minimum Information About a Plant Phenotyping Experiment) standard.

Results: The FruitRescue database is introduced here, together with an overview of the project's scope and the network of common garden sites set up in diverse environments. Focusing on apple trees as a case study, we describe the experimental design and the phenological and phenotypic traits currently available in the database. We also share preliminary results that could highlight potential patterns of local adaptation within this framework.

Conclusion

This unprecedented database will serve as a valuable resource for the scientific community, enhancing our understanding of how fruit trees respond to climate change in both wild habitats and agricultural systems. It will also provide crucial guidance for breeders seeking to develop climate-resilient varieties and for conservation managers working to protect the wild relatives of fruit trees.



Genomic assessment of existing resources and climate maladaptation risk in Scots pine: implications for forest management

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POSTER SESSION 1 - **P01.010**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

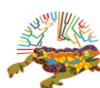
Background: Forest trees profoundly influence ecosystem functioning, biodiversity, and climate stability globally. Due to their longevity, broad distribution, and stationary nature, trees harbor extensive genetic variation shaped by historical climatic fluctuations and local adaptation processes. Among these, Scots pine (*Pinus sylvestris* L.) stands out as a widespread and ecologically significant conifer across Eurasia. It displays a complex genetic architecture shaped by past demographic processes and present-day climatic gradients. In the context of accelerating climate change, understanding the genomic basis of local adaptation is essential for predicting how tree populations will respond and for guiding proactive forest management strategies, such as assisted migration and targeted seed sourcing.

Methods: We investigated patterns of genomic diversity and local adaptation in 76 Scots pine populations (N = 1281 individuals) spanning a broad latitudinal range across Europe. Using high-density SNP arrays and mitochondrial DNA (mtDNA) markers, we examined population structure and historical divergence across the species' range. Subset of samples along steep latitudinal gradient was used for environmental association analyses, including redundancy analysis (RDA), to identify genomic loci associated with key climatic factors, especially mean annual temperature. To assess future risks of maladaptation, we performed genomic offset analyses using both RONA and RDA-based approaches, projecting adaptive mismatches under future climate scenarios.

Results: Our analyses revealed the existence of two major genetic lineages, which diverged during the mid-Pleistocene. Remarkably, these lineages have persisted despite ongoing gene

flow via effective pollen mediated gene flow. However, they did not reflect the strict division of the population into the analyzed seed zones of the species. We identified numerous SNPs strongly associated with temperature, indicating robust local adaptation across environmental gradients. Notably, populations in Central and Northern Europe exhibited heightened genomic differentiation at adaptive loci, pointing to region-specific selection pressures and increased vulnerability to future climatic shifts. Genomic offset modeling highlighted considerable risks of future maladaptation, particularly for populations located at ecological and geographical range margins. Our results indicate that adaptive genetic shifts required to cope with projected climate scenarios may require centuries through natural selection alone, emphasizing significant adaptive constraints.

Conclusions: Our study demonstrates the coexistence of deep historical genetic structure and clear signatures of contemporary adaptation within Scots pine. The identification of climate-associated genomic markers provides valuable tools for climate-resilient forest management. Specifically, these markers can inform assisted gene flow, provenance trials, and selection of planting material suited to future climates. Importantly, our findings advocate for the integration of genomic data into conservation policies, to safeguard the long-term adaptive potential and ecological resilience of forest ecosystems. As environmental change accelerates, such genomic-informed strategies will be indispensable in sustaining the biodiversity and functional integrity of temperate forests across Europe.



Genomic Signatures of Drought Adaptation in Eucalyptus: A Phylo-transcriptomic Approach Across 50 Million Years of Divergence

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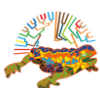
POSTER SESSION 1 - P01.008, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

As climate change intensifies drought conditions worldwide, understanding the genomic basis of drought tolerance becomes increasingly critical for forest conservation and management. Eucalyptus presents an exceptional system to study adaptation to aridity, with species distributed across Australia's diverse climate gradient, from mesic to arid environments. This distribution represents multiple independent evolutionary transitions into drought-prone habitats, providing a natural experiment in convergent evolution.

Our study employs a phylo-transcriptomic approach to investigate whether repeated adaptation to drought has utilized similar or different genetic pathways across phylogenetically distant species pairs. We selected eight taxonomic sections spanning three major Eucalyptus subgenera, with each section represented by closely related species pairs from contrasting environments. Using a robust common garden experimental design, we subjected seedlings to controlled drought-recovery cycles and measured transcriptional responses alongside physiological parameters.

Dimension reduction analyses reveal that gene expression patterns, rather than genomic content, primarily characterize species differences in drought response with gene usage clustering according to phylogeny and climate of origin. Using novel Bayesian phylogenetic comparative methods specifically developed for transcriptome data, we formally incorporate phylogenetic relationships into gene and gene-isoform expression analysis, allowing us to detect convergent and divergent transcriptomic signatures of drought adaptation. This unified statistical framework permits estimation of phylogenetic signal for each gene while coupling across the transcriptome using regularization methods.

This study examines the genomic basis of adaptive resilience to water stress and addresses fundamental questions about the predictability of evolution: Do species repeatedly rediscover the same molecular mechanisms when facing similar environmental challenges, or do they find unique solutions? These insights contribute both to evolutionary theory and practical applications in developing strategies to maintain forest resilience under climate change.



Genotype–environment interactions underlying resistance to *Hymenoscyphus fraxineus* in European ash

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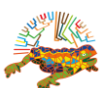
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POSTER SESSION 1 - **P01.009**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The invasive fungal pathogen *Hymenoscyphus fraxineus* (HF) continues to cause widespread declines in European ash (*Fraxinus excelsior* L.) populations across Europe. Understanding the genomic and environmental determinants of resistance is essential for developing adaptive management strategies, particularly as climate change may alter disease dynamics. In this study, we analyzed whole-genome sequencing data from 320 ash trees sampled across 109 populations in Poland, representing a broad range of ecological and climatic conditions. A genome-wide association study (GWAS) identified 114 SNPs significantly associated with resistance to HF, with over 40% located on chromosome 14, suggesting a potential genomic hotspot under selection. Genomic prediction models based on Random Forest and subsets of $\geq 2,500$ SNPs achieved over 80% accuracy in predicting

tree health status, with peak performance (~91%) observed for extreme phenotypic classes. Incorporating bioclimatic variables—particularly those related to temperature and precipitation seasonality—further improved prediction accuracy by up to 8%, especially when training and testing occurred within similar environments. In contrast, model performance declined when applied across divergent environmental contexts, highlighting the complexity of genotype-by-environment (G×E) interactions.

Our findings underscore the power of integrating genomic and environmental data to elucidate the adaptive architecture of disease resistance in *F. excelsior*. This integrative approach offers valuable applications for resistance-informed breeding, targeted conservation, and enhancing forest resilience to emerging biotic threats.



Islands as model systems for evolutionary divergence: a case study of *Hypericum lanceolatum* on Réunion Island

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POSTER SESSION 1 - **P01.007**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

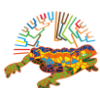
Islands are increasingly recognized as model systems to study evolutionary processes. Their geographical isolation from continental coasts promotes genetic divergence and the development of distinct evolutionary lineages. Young volcanic islands, in particular, with their rugged topography and high relief, offer a multitude of ecological niches that lead to high diversification and speciation. As such, islands like Réunion and Hawaii are renowned for their remarkable radiation and extreme polymorphism across various taxa. Understanding the molecular underpinnings of divergence in insular species is therefore critical to ensure the long-term conservation of island biodiversity.

Here, we explore morphological and molecular divergence patterns in *Hypericum lanceolatum*, an indigenous shrub endemic to Réunion Island. Two botanical subspecies are currently described: *Hypericum lanceolatum* subsp. *lanceolatum* from 550 to 1700m and *Hypericum lanceolatum* subsp. *angustifolium* from 1800 to 2900m. The large variation exhibited in *H.*

lanceolatum morphology across the elevational gradient raises basic questions: What traits most distinctly differentiate these morphotypes? To what extent do genetic divergence and environment drive these differences?

To address these questions, we conducted extensive field sampling across the island to encompass the full morphological range of the species. Molecular divergences were assessed by genotyping-by-sequencing, and phenotypic plasticity was evaluated through a common garden experiment. Our results reveal a continuum of genetic differentiation along the elevational gradient, consistent with current taxonomy delimitation. However, morphological traits showed high plasticity under controlled conditions, suggesting a strong influence of environmental factors.

This work provides a valuable framework for studying evolutionary responses to environmental gradients and has implications for the conservation of island endemic species under changing climatic conditions



Landscape Genomic Tool built on genotype-environment associations of truly wild olive trees sampled along a 13-degree north-south in the Western Mediterranean to Assess the Adaptive Value of Cultivars

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POSTER SESSION 1 - P01.012, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Crop wild relatives and landraces represent locally adapted germplasm, enabling landscape genomic studies to identify genomic signatures of environmental adaptation. The derived information from such studies can be used to evaluate cultivar's adaptive value. This approach can represent an advantageous opportunity particularly for perennial crops where multi-location field trials to test specific cultivar or new breeding clone adaptations are costly and time-consuming.

We applied this approach to olive trees (*Olea europaea* subsp. *europaea* var. *sylvestris*), leveraging truly local wild germplasm from 27 populations sampled along a 13-degree north-south gradient, covering regions in Southern France, Corsica, Spain, and Morocco.

Using capture sequencing data targeting more than 50 000 predicted genes, we identified candidate polymorphisms linked to climate and soil variables through genotype-environment associations (GEAs), allowing us to map the adaptive genetic variation of olive trees across the estimated species range in the western Mediterranean basin. These findings can inform sampling recommendations aimed at capturing wild adaptive diversity.

Building on these GEAs, we applied the RDA framework to predict the spatial genomic

offset of new genotypes, which in this case were represented by widely used cultivars of the domesticated olive tree (*Olea europaea* subsp. *europaea* var. *europaea*). For a set of cultivars, the prediction allowed to identify regions where adaptation could enhance local performance and reduce reliance on agricultural inputs. The model confirms a latitude-based correspondence between specific cultivars' growing environments and adaptive regions in the western Mediterranean. Additionally, it highlights the broad adaptive-genetic variation present in western wild genotypes, which is currently underrepresented in cultivated germplasm, emphasizing the need for conservation strategies and breeding utilization.

Given the co-occurrence of wild and cultivated populations in the same area, and the effective one-way gene flow from vegetatively propagated cultivars to sexually reproducing wild populations, we could pinpoint areas where the cultivar offset is minimal. In these regions, the coexistence of wild and cultivated genotypes is less likely to disrupt the GEA pattern thereby preserving the integrity of wild adaptive diversity.

Collectively, these findings offer valuable insights for developing in-situ conservation strategies for wild relatives and informing breeding programs to enhance environmental adaptation.



Local adaptation and Genomic vulnerability to climate change in oak species

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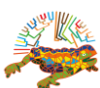
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POSTER SESSION 1 - **P01.005**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Understanding the genetic basis of local adaptation and its influence on species' responses to future climate change is crucial for forest conservation. We integrated pan-genomic and environmental association analyses to investigate adaptive evolution in two widespread East Asian oaks, *Quercus variabilis* and *Q. acutissima*. High-quality genome assemblies of 22 ecogeographically representative accessions revealed 543,372 structural variations (SVs), highlighting their critical roles in shaping genomic structure and influencing gene expression. Genotype-environment association analyses identified polygenic adaptation patterns, with climate-associated single nucleotide polymorphisms (SNPs) and SVs distributed across distinct functional gene categories. Notably, SV-based selection signals showed minimal overlap with SNP outliers, suggesting that conventional SNP-centric approaches may overlook key adaptive elements. A significant finding was a 250-kb adaptive introgression hotspot on chromosome 9 (Chr9-ERF), containing eight ethylene-responsive factor (ERF) genes and enriched candidate SNPs. Population genomic evidence confirmed westward introgression of advantageous haplotypes from *Q. acutissima* into *Q. variabilis*, providing mechanistic insights into how interspecific gene flow enhances local adaptation.

To quantify climate vulnerability, we applied a tripartite genetic offset framework to assess genomic-environmental decoupling under future climate scenarios. Key findings include: 1) Marginal populations exhibited elevated extinction risks due to limited habitat connectivity and dispersal constraints; 2) Northern China showed disproportionately high reverse genetic offsets, indicating insufficient adaptive potential of extant genetic variation; 3) Climate-driven range shifts may disrupt established genotype-environment correlations even in currently well-adapted populations.

This study advances three critical dimensions of evolutionary conservation biology: First, it establishes SVs as indispensable components in deciphering adaptive landscapes, complementing traditional SNP analyses. Second, it demonstrates that interspecific introgression serves as an evolutionary "accelerant" for climate adaptation in long-lived trees. Third, our multidimensional offset model provides actionable metrics for prioritizing conservation efforts. By revealing how historical selection, standing variation, and gene flow jointly shape climate resilience, this work offers a genomic roadmap for predicting and mitigating anthropogenic climate change impacts on keystone forest species.



Multi-Omics Insights into Seasonal Adaptation and Resilience in *Prosopis cineraria*

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POSTER SESSION 1 - **P01.001**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

In the face of accelerating climate change and increasing aridity, desert ecosystems serve as vital models for biological resilience. *Prosopis cineraria* (Ghaf tree), a legume native to hyper-arid regions of the Arabian Peninsula and South Asia, exemplifies this resilience as a keystone species. However, the molecular basis of its adaptive resilience remains largely unexplored. This study presents the first integrative seasonal multi-omics characterization of *P. cineraria*, combining genomic, transcriptomic, epigenomic, and phenotypic analyses to dissect two principal adaptive traits: carbon assimilation and flowering regulation. Our results highlight a C₃-C₄ intermediate photosynthetic strategy that becomes especially relevant during the peak heat season. This intermediate pathway is supported by the upregulation of PEPC2 and GLK1, among other genes, whose expression colocalizes with seasonally dynamic topologically associating domains (TADs) in the chromatin architecture—suggesting a higher-order regulatory integration of photosynthetic and thermal adaptation. Moreover, we observe a novel co-regulatory interaction between reproductive phenology and carbon assimilation. Epigenomic profiling of

flowering regulation demonstrates that *P. cineraria* exhibits biannual flowering, occurring from March to May and from September to November, each governed by distinct molecular pathways. Spring flowering proceeds via a vernalization-responsive mechanism, wherein CpG methylation silences VRN1, thereby derepressing VRN2 and VRN3, repressing FLC, and ultimately activating FT–FD-driven floral initiation. In contrast, autumnal flowering is regulated through an autonomous pathway marked by the enrichment of H3K4me3 and H3K27ac histone modifications at the FCA locus, promoting FLC repression in the absence of cold cues. This dual flowering strategy, combined with the C₃-C₄ photosynthetic strategy, underscores a sophisticated degree of temporal plasticity, enabling reproductive success across seasonally variable climates. Our study elucidates the genetic and epigenetic architectures underlying survival in hyper-arid environments and provides foundational genomic resources with translational potential for resilience breeding in C₃ leguminous crops, which offers an essential framework for sustainable adaptation in the Anthropocene.



Towards an integrative understanding of taxonomic diversity, climate adaptation, and introgression in Southeastern European white oak species

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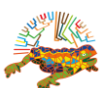
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POSTER SESSION 1 - P01.003, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Oaks (genus *Quercus* L.) are considered potential winners under future drier and warmer climates. The white oak species complex includes some of the most widespread oak species in Europe – *Q. robur*, *Q. petraea*, and *Q. pubescens* – and is strikingly diverse in Southeastern Europe, where a number of subspecies have been reported. However, the evolutionary processes underlying this taxonomic richness remain unclear. We hypothesize that climate adaptation and adaptive introgression during the divergence of white oak lineages play a major role. Our project features whole-genome resequencing (WGR) and leaf morphological data from over 70 populations sampled along a transect spanning from southern Switzerland and Northern Italy, through Southeastern Europe and Turkey to the Caucasus, including several white oak diversity hotspots. Sampling design includes pure and mixed-species stands, allowing us to disentangle ancient from recent hybridization and define the genetic composition of each lineage. With this dataset, we aim at investigating the demographic history and the extent and direction of gene flow among white oak lineages. To investigate the role of gene flow in driving local adaptation, we will identify candidate loci for climate adaptation

through genotype-environment association studies using publicly available climatic data, and test whether they are involved in hybridization, pointing to adaptive introgression. We will also focus on populations at the ecological range margins to assess white oak adaptive potential under future climates. Ongoing WGR analyses show clear genetic differentiation between species, but not among subspecies. The extent of introgression among species appears to vary among chromosomes, indicating a non-random distribution of introgressed material that is maintained in the population. Leaf morphology differentiates putative hybrids and species but struggles with minor lineages due to large within-species variation. These results suggest different stages of differentiation among white oak lineages, allowing for investigation into local adaptation on multiple evolutionary scales. This large-scale study combining genomic, environmental and phenotypic data will significantly contribute towards a better understanding of taxonomic richness and adaptive potential in white oaks, and in hybridizing, sympatric species complexes in general.



A higher load of deleterious mutations has a detrimental effect on tree growth

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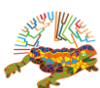
MEETING ROOM 122+123, August 18, 2025, 10:30 AM - 12:30 PM

A high proportion of deleterious mutations in the genome is predicted to cause a reduction in fitness known as genetic load. In plants, most studies focus on how detrimental mutations increase as a consequence of population bottlenecks, for instance in cultivated species as a negative outcome of domestication or at the margin of species ranges. However, the actual adverse effect of genetic load on individual phenotypes remains little characterised, especially in wild plant species with large populations and long lifespans.

We estimated genetic load based on genomic data in populations of the conifer maritime pine (*Pinus pinaster*, Ait.) on the island of Corsica (two different datasets including 133,656 SNPs in 393 individuals and 10,185 SNPs in 334 individuals) and examined its association with phenotypic traits related to growth, phenology, and

water-use efficiency measured under common garden conditions. Deleterious genetic variants were predicted from genomic sequencing data using classic (SIFT, PROVEAN, SNPEff) and Artificial Intelligence-based (ESM-1v) methods.

Our results revealed a negative correlation between genetic load and tree height on the Mediterranean island of Corsica, thus suggesting a negative cumulative effect of deleterious mutations on tree growth at the regional scale. This association was further confirmed by a wide-range analysis including 464 individuals from 34 populations across the entire range of maritime pine in the western Mediterranean, Atlantic Europe, and North Africa. Our study is one of the first to experimentally demonstrate adverse phenotypic effects (reduced tree growth) of genetic load in a long-lived plant.



Adaptation to ash dieback disease in natural populations of European ash

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MEETING ROOM 122+123, August 18, 2025, 10:30 AM - 12:30 PM

Natural tree populations are exposed to increasing threats from biotic and abiotic stressors. One recent case with large predicted ecological and economic consequences is ash dieback (ADB), a fungal disease currently decimating ash (*Fraxinus excelsior*) populations in the UK and northern Europe.

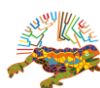
Although there is some evidence that European ash populations are already adapting to the disease via selection on standing genetic variation, it is still unclear 1) if this process will be fast enough to conserve populations and 2) if increased mutation rates in response to stress could generate resistance alleles quickly enough for adaptation.

Additionally, while nearly all plant adaptation studies to date have focused on small genetic variants, recent advances in long read sequencing are allowing the investigation of large structural variants (SVs) with potentially larger phenotypic effects. Under stressful conditions, processes such as transposon reactivation or increased recombination may also quickly generate large amounts of this type of genetic variation.

To investigate these questions and predict the prospects for ash in northern Europe, we phenotyped and sequenced three UK

populations, generating Illumina short-reads for approximately 950 trees born either pre-epidemic (adults > 30 years) or during-epidemic (juveniles < 7 years). We then assigned parentage and generated PacBio HiFi reads for approximately 300 trees between known parents and their offspring.

By means of Genome Wide Association, we screened for small variants associated with ADB resistance/susceptibility, using visual phenotypic assessments of disease severity and also the quantification of fungal DNA load from the short reads. Despite the well-known strongly polygenic nature of ADB resistance we identified alleles associated with disease damage and are now testing for 1) allele frequency shifts and 2) differences in mutation rates, before and after the arrival of the epidemic. Using HiFi reads, we are calling SVs in a pangenome framework to identify additional genetic bases for resistance and novel SVs in parent-offspring pairs. To address the mechanisms that may be responsible for the generation of novel SVs, we have also produced a curated transposable element annotation, that will allow us to pinpoint which SVs are the result of transposition vs other processes. All together this will provide fresh insights into how natural populations adapt to novel selective pressures.



Genomic signatures of climate-driven (mal)adaptation in an iconic conifer, the English yew (*Taxus baccata* L.)

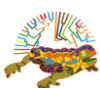
Francisco T¹, Mayol M², Vajana E³, Riba M^{2,4}, Westergren M⁵, Cavers S⁶, Pinosio S³, Bagnoli F³, Marchi M³, Aravanopoulos F⁷, Farsakoglou A⁷, Scotti I⁸, Fady B⁸, Vendramin G³, Piotti A³, Archambeau J¹, González Martínez S¹

¹National Research Institute for Agriculture, Food and the Environment (INRAE), University of Bordeaux; BIOGECO, ²CREAF, ³Institute of Biosciences and BioResources (IBBR), National Research Council (CNR), ⁴Universitat Autònoma de Barcelona, ⁵Slovenian Forestry Institute, ⁶UK Centre for Ecology & Hydrology (UKCEH), ⁷Laboratory of Forest Genetics, Faculty of Agriculture, Forestry and Natural Environment, Aristotle University of Thessaloniki, ⁸National Research Institute for Agriculture, Food and the Environment (INRAE), URFM, Ecology of Mediterranean Forests

MEETING ROOM 122+123, August 18, 2025, 10:30 AM - 12:30 PM

The risk of climate maladaptation is increasing for numerous species, including forest trees. Developing robust methods to assess population maladaptation remains a critical challenge. Genomic offset approaches aim to predict climate maladaptation by characterising the genomic changes required for populations to maintain their fitness under a changing climate. In this study, we assessed the risk of climate maladaptation in European populations of English yew (*Taxus baccata*), a threatened, long-lived forest tree species widely distributed in small patchy populations across Europe, the Atlas Mountains and the Near East. First, we found evidence of local adaptation to climate across populations by analysing 8,616 SNPs genotyped in 475 trees from 29 populations across the European *T. baccata* distribution, with 18.1% of genetic variance explained by climate and the identification of 100 unlinked climate-associated loci using genotype-environmental association (GEA). Second,

we evaluated the deviation of populations from the overall gene-climate association to assess variability in local adaptation or different adaptation trajectories across populations. Third, we predicted genomic offsets and successfully validated these predictions using fitness proxies from a nearly independent dataset of 26 populations planted under common garden conditions. Finally, we integrated information from current local adaptation and the genomic offset, with historical genetic differentiation and effective migration rates to show that high-altitude *T. baccata* populations face higher future vulnerability to climate change than coastal or other low-altitude populations. Our study demonstrates the practical use of the genomic offset framework, provides insights to improve it and highlights the need for a population-centred approach that incorporates additional statistics and data sources to assess credible climate vulnerability in wild plant populations.



When South meets North: the shaping of a contact zone in three boreal tree species

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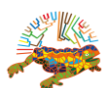
Climatic oscillations during the Quaternary, particularly the Last Glacial Maximum (LGM, ~18,000 years ago), induced repeated expansions and contractions of species ranges, thereby shaping the current distribution of European species. During the LGM, most of northern Europe was completely covered in ice, leading to the isolation of several species in refugia located south or east of the ice sheet. As global temperatures increased and the ice sheet receded, populations from various refugia recolonized northern Europe, thus establishing extensive contact zones where previously isolated lineages meet. These contact zones, along with the analyses of genome-wide markers, provide information on unique genetic admixture patterns and offer important insights into the divergence history of populations.

A recent contact zone in central Scandinavia has been identified for several organisms, and its study has recently been extended to forest tree species. In this project, we perform a comparative analysis of the three most common forest tree species in Scandinavia: *Picea abies*, *Betula pendula* and *Pinus sylvestris*. First, we identified similar demographic histories, but varying levels of gene flow across species, with *P. sylvestris* showing the highest levels and *P. abies* the lowest. Most notably, we demonstrate the presence of two main genetic clusters within each studied species, overlapping in a shared contact zone between 60° and 63°N. This contact zone

also corresponds to the climatic transition between the two major environmental zones in Sweden, suggesting a role for environmental factors in maintaining the two main genetic clusters of these species.

Furthermore, we detected genomic barriers to gene flow and identified signatures of selection likely driven by adaptation to contrasting environments. Finally, we show that differences in gene flow levels lead to species-specific patterns of adaptive genetic architecture, providing empirical support for theoretical expectations about adaptation under gene flow. Thus, we conclude that a contact zone in central Sweden is present in the three species and is maintained by natural selection, despite ongoing gene flow between the two main genetically divergent lineages of each species.

In general, contact zones have proven to be a major source of information on past demography and the selection pressures undergone by different populations or taxa. Together with genetic data, they provide insight into the evolutionary mechanisms that maintain barriers to gene flow and the origin of current genetic diversity. We also highlight that the study of contact zones in forest tree species is crucial for predicting how natural populations may respond to ongoing climate change, provides a comprehensive view of the genetic composition of species, and allows the refinement of sophisticated breeding strategies based on genomic predictions.





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S02 - Addressing new and long-standing evolutionary questions with linkage disequilibrium based approaches



A Linkage-Based Method to Detect Introgression in Polyploid Genomes

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POSTER SESSION 3 - P03.005, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

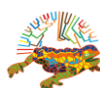
The detection of introgression in polyploid genomes remains a major challenge for evolutionary and genomic research, especially when working with short-read data. Most existing methods are designed for diploid organisms or rely on assumptions and resources, such as accurate phasing, that remain difficult to achieve in polyploid systems. To address this, we developed a linkage-based method that uses correlations among variants within genomic windows to detect ancestry and identify introgressed regions in polyploid species.

We applied this method to *Solanum tuberosum*, the cultivated tetraploid potato, which has a complex and only partially resolved evolutionary history. Numerous wild *Solanum* species, spanning a wide geographic range from Mexico to Argentina, are potential contributors to its genome. Introgression is believed to have taken place during both the initial Andean domestication and subsequent breeding. However, the identity and genomic impact of specific donor species remain poorly understood.

Our dataset included 827 whole-genome resequenced samples, encompassing a global diversity of 166 cultivars and

representatives from 78 wild species. Ancestry blocks were identified by clustering variants in linkage disequilibrium, allowing us to trace their origins to specific wild donors along the genome. We found pervasive but heterogeneous introgression across both chromosomes and cultivars, with certain wild species making disproportionately large contributions. We found that introgression from wild species strongly predicts levels of genetic diversity in cultivated genomes, underscoring its central role in shaping the genomic landscape of potato.

Despite this extensive admixture, introgressed segments predominantly cluster into a limited number of haplotypes, indicating reduced haplotype diversity despite the presence of multiple potential donor species. This pattern points to one or a few recent introgression events for most wild species, as evidenced by the extended sizes and recurrence of shared haplotype blocks. Overall, this framework provides a practical and scalable solution for studying ancestry in polyploids and offers insights relevant to crop breeding and evolutionary genomics.



Asymmetric gene flow and phenotypic clines across a European butterfly hybrid zone

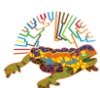
Cama B¹, Decroly T², Fisher A³, Vila R⁴, Baird S⁵, Lohse K²

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POSTER SESSION 3 - P03.014, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Hybrid zones are an important tool in evolutionary biology. The genomes of naturally occurring hybrids can reveal the direction of gene flow and the presence/absence of genomic regions that remain divergent in the face of widespread hybridization, so-called islands of divergence. Two European butterfly sister species, *Melanargia lachesis* and *Melanargia galathea*, differ in distribution and in phenotype but overlap in range within a narrow hybrid zone in the Pyrenees, where hybridization is frequent. Using a pilot dataset of wild-caught hybrids from this region, we systematically quantify phenotypic differentiation and gene flow between species. We achieve this via a novel approach, by using the state-of-the-art diem software to attribute ancestries to each

variant in the hybrid zone *Melanargia* genomes and produce a SNP-level genomic admixture map across different individuals. We overlay this information with a landmark-based quantitative analysis of wing size and colour pattern, and find wing phenotype to be strongly correlated with chromosome-level hybridization indices, potentially showing a strongly multigenic architecture for the measured traits. Hybridization patterns show that the direction of gene flow in the hybrid zone is asymmetric, favouring introgression from *M. galathea* into *M. lachesis* more so than the opposite. This is the first investigation into this hybrid zone's population genetics and lays the methodological foundations for future research into this system as well as similar ones.



Demographic inference given rampant background selection under a high deleterious mutation rate

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*POSTER SESSION 3 - **P03.010**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM*

Linked background selection (BGS) is well known to reduce genetic diversity in a population via selection against deleterious alleles at linked loci. Relative to this phenomenon, unlinked BGS is widely considered to be negligible. It is thus frequently omitted from population genetics simulations, especially considering that the inclusion of unlinked BGS means tracking the whole genome rather than more efficiently examining only a smaller genomic window. Recent research by Matheson and Masel (2024) has shown, however, that under a realistically high deleterious mutation rate, unlinked BGS is not only significant, but it has a greater impact on neutral diversity than linked BGS. Hence, failing to include unlinked BGS may limit the accuracy of such simulations. This is particularly true when it comes to inferring

the demographic history of a population in the wake of a brief genetic bottleneck using its site frequency spectrum (SFS), which is a graphical depiction of the distribution of allele frequencies across the genome. Bottlenecks tend to increase the prevalence of recessive deleterious homozygotes, causing prolonged, intensified BGS. As a result, the SFS may mimic that associated with an extended population size reduction, even in the case of rapid regrowth, thanks to an enrichment for common variants rather than rare ones. My research aims to utilize the Masel lab's whole-genome forwards time simulation to investigate the demographic inference of a brief bottleneck under a realistically high deleterious mutation rate, considering how the inclusion of unlinked BGS may impact the SFS.



Ecological speciation in North Atlantic Redfish (Sebastes): Insights from whole-genome data

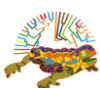
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¹Fisheries And Oceans Canada

POSTER SESSION 3 - P03.007, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Redfish (*Sebastes* spp.) in the North Atlantic offer an opportunity to study ecological speciation in a marine environment. While their Pacific relatives have undergone a massive radiation, only four species — *S. fasciatus*, *S. mentella*, *S. norvegicus*, and *S. viviparus* — occur in the North Atlantic, often occurring in sympatry. The genus contains numerous species that are depth-segregated, and along with cryptic morphology and complex life histories, present both evolutionary questions and fisheries management challenges. Using whole-genome sequencing, we explored the evolutionary history of these species and

examined genetic diversity, population differentiation and linkage disequilibrium (LD) to understand the diversification of redfish in the North Atlantic. We characterise hierarchical and ecological genetic divergence in North Atlantic redfish and identify heterogeneous signals of post-glacial secondary contact between nuclear and mitochondrial genomic data. We connect the importance of identifying the genetic basis underlying divergence of redfish to both evolutionary biology and sustainable resource management as climate change continues to alter ocean conditions.



Estimating very recent population split times using decay of IBD (identity by descent) sharing

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POSTER SESSION 3 - **P03.011**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Standard models for estimating divergence times between populations suffer from several modeling shortfalls, including an inability to distinguish between genetic drift caused by small population sizes or longer divergence times, and the reliance on an unrealistic assumption of random mating within a population. Here we present a new method for estimating recent divergence times that utilizes information in shared identity-by-descent (IBD) segments within and between populations, and which is insensitive to assumptions about random mating or population size. IBD sharing can be thought of as an extreme form of linkage

disequilibrium caused by the shared inheritance of an extended haplotype. We apply our approach to whole-genome sequence data from Indian caste groups sampled from the Birbhum district in West Bengal (as part of the GenomeAsia 100K project), and estimate average divergence times (i.e., onset of endogamy) of ~10-20 generations ago between different self-identified Hindu caste groups. This is much more recent than previous estimates, and highlights the complex structure of population assimilation/mixing that highlights most of our species' history.



Filtering linked selection to improve LD-based demographic inference in heavily exploited Baltic cod (*Gadus morhua*) populations

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POSTER SESSION 3 - **P03.008**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

A central assumption in population genomics is that selection affects local genomic regions while demography leaves global signatures. However, widespread linked selection—through background selection and genetic hitchhiking—can affect linkage disequilibrium (LD) patterns across the genome, biasing demographic inference and challenging this assumption. This issue is especially pronounced in systems under long-term directional selection, such as fisheries-induced evolution (FIE) in Eastern and Western Baltic cod, where the length at which 50% of Eastern Baltic cod females reach maturity has declined by over 40% since the 1990s, from ~40 cm to ~23 cm. LD provides insight into recent changes in effective population size (N_e), as it decays over time with recombination and thus reflects recent coalescent history. However, demographic models assume that genome-wide LD is shaped by genetic drift rather than selection, which is violated by strong selection --such as FIE -- that maintains long haplotypes and inflates LD independently of drift, thereby biasing N_e estimates. To address this, we apply extended haplotype homozygosity (EHH) based scans, which detect recent positive selection by identifying unusually long homozygous haplotypes indicative of

reduced recombination around selected alleles. We enhance the resolution of these scans by using Oxford Nanopore Technologies (ONT) long-read sequencing to improve haplotype phasing, allowing precise detection of selected regions in heavily exploited cod populations. By filtering out regions under recent selection, we aim to reconstruct recent N_e trajectories shaped primarily by demographic processes, anticipating a signal of the population collapse in the 1990s and later on partial recovery. As an additional goal, we investigate the dynamics of gene deletion polymorphisms in an Eastern Baltic cod time-series genomic dataset spanning from 1996 to 2025, some of which show preliminary associations with traits like reduced body size and circadian regulation. Overall, this work provides evidence of genome-wide responses to large-scale exploitation, and will hopefully allow us for a more accurate reconstruction of demographic history aware of pervasive selection and structural variation. Our framework may be broadly applicable to other wild populations affected by strong directional selection, highlights the need to consider the influence of genome architecture to demographic frameworks.



Historical divergence and secondary contact shape the distribution of genetic variation in a key glacial refugium

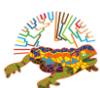
Gentile G¹, Franchini P¹, Momigliano P¹, Chiocchio A¹, Canestrelli D¹

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POSTER SESSION 3 - P03.013, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The Italian Peninsula, a major glacial refugium characterized by hotspots of intraspecific genetic diversity, represents a region of critical importance for conserving the adaptive potential of wildlife populations. Its southernmost region, the Calabrian Peninsula, harbours the highest levels of genetic diversity for several taxa, including *Rana italica*, a species endemic to the Apennine mountain ranges. Preserving the evolutionary potential of such taxa requires a detailed understanding of spatial patterns of genetic variation, which often reflect complex demographic histories. For example, following historical isolation, secondary contact zones can present elevated genetic diversity, a result of introgression between divergent lineages. Using genome-wide data, we investigated the genetic structure and diversity of *Rana italica* populations within and around Aspromonte National Park, located at the

southern tip of the Calabrian Peninsula. Despite the limited geographic scale of sampling, population structure analyses revealed two distinct genetic clusters and one admixed group. The admixed group has the highest levels of heterozygosity, yet much stronger Linkage Disequilibrium (LD) than the other two less diverse populations, a pattern characteristics of secondary contact zones. Demographic reconstructions, including LD-based approaches, also support a scenario of recent secondary contact. Our findings underscore the value of integrating population genomic and demographic analyses to inform conservation strategies, particularly in regions with complex biogeographic histories and high endemism. Such approaches are essential for the effective management and preservation of evolutionary processes in fragmented habitats.



Identification of novel toxin resistance genes in *Drosophila* via Experimental Evolution, GWAS, and CRISPR screening

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POSTER SESSION 3 - P03.003, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Insecticide resistance is a widespread challenge for the management of vectors transmitting pathogens and agricultural pests, requiring a better understanding of the genetic mechanisms underlying evolution of resistance. *Drosophila sechellia* is a compelling model for such studies as it naturally evolved resistance to octanoic acid (OA), an abundant chemical of its noni fruit host that is toxic for other insects, including close relatives *D. simulans* and *D. melanogaster*. We have used a multi-pronged strategy to identify genes contributing to OA resistance. We began by experimentally-evolving *D. simulans* strains with higher tolerance to OA, and determined the resulting genetic architecture. To identify specific candidate genes, we integrated this analysis with a genome wide association study of OA resistance in *D. simulans* as well as a genome-wide CRISPR selection screen upon OA exposure in *D. melanogaster* S2R+ cultured cells. We identified four candidate genes with diverse predicted molecular and

expression properties and validated their relevance through RNAi-mediated knockdown and transgene-mediated overexpression in *D. melanogaster*. Three of these genes increased OA susceptibility when knocked down, while the fourth promoted *D. sechellia*-like levels of OA resistance. Notably, two genes showed elevated expression in experimentally evolved strains, mirroring their higher expression in *D. sechellia* compared to other drosophilids. Population genetics analyses further suggest signs of positive selection in at least one gene's coding region. Finally, we examined the effects of loss-of-function mutations in *D. sechellia*. Our results hint at an adaptive role of these genes in shaping OA resistance both under laboratory conditions and during *D. sechellia*'s evolutionary history. This study emphasizes the power of integration of multiple genetic approaches to identify genes and cellular mechanisms underlying toxin resistance in insects.



Inferring population size history from both modern and ancient genomes using approximate bayesian computation

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*POSTER SESSION 3 - **P03.009**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM*

One of population genetics oldest challenges is the inference of effective population size (N_e), a central parameter that impacts the genetic diversity and resilience of a population. Population and conservation genetics are keenly interested in its estimation. The constant improvement of sequencing methods and the quickly growing amount of available genetic data have motivated the development of methods capable of screening vast quantities of whole-genome data to estimate past N_e trajectories for thousands of generations. Such algorithms include Sequentially Markovian Coalescent (SMC) approaches such as the PSMC (Li & Durbin, 2011) and MSMC (Schiffels & Durbin, 2014), but also methods based on Approximate Bayesian Computation (ABC, Tavaré et al. 1997). ABC methods use summary statistics to infer N_e , which allow them to take into account whole-genome data such as Single Nucleotide Polymorphism (SNP). The SNPs' abundance (how often a SNP is present in a population), their allele frequency distribution and linkage disequilibrium (how

allele frequencies at two SNPs are correlated) are good indicators of past population size. However, all those methods use modern DNA samples for their calculations. Ancient DNA, or DNA extracted from archeological samples, is a potential way to improve past N_e estimates by giving us a glimpse in the population' past genetic diversity. To test this hypothesis, we updated PopSizeABC (Boitard et al. 2016), an ABC method using whole-genome SNP data to infer past N_e trajectories, so that it can take into consideration DNA data from several time points. Using simulated modern and ancient DNA under various population' histories, we show that the addition of new summary statistics accounting for allele frequency variations across generations reduces prediction error. In the future, we plan to apply PopSizeABC to empirical data, using the European Eel (*Anguilla anguilla*) as a test species. The European Eel, being a species supposed to be panmictic, is an especially interesting model as both ancient and modern samples can be assumed to come from the same population.



Investigating the role of meiotic recombination in social insects - Termites adding an important piece to the puzzle

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¹Uppsala University, ²University of Freiburg, ³Charles Darwin University

POSTER SESSION 3 - **P03.004**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Meiotic recombination is an essential process in all sexually reproducing organisms as it increases the efficiency of selection. There is however great variation in recombination rate across the tree of life, with eusocial insects from the order Hymenoptera in the topmost range. An example is the honeybee which has recombination rate estimates ranging between 19-37 cM/Mb. It has been hypothesized that eusociality leads to selection for high recombination rates as this would increase the genetic diversity in colonies and reduce kin conflict. The association between eusociality and high recombination rate has however not previously been studied outside of Hymenoptera.

Termites belong to the order Blattodea, which is separated from Hymenoptera by 380 million years. As termites are eusocial insects, they are expected to have high recombination rates according to the aforementioned hypothesis. In this study we have sequenced 20 samples from two termite species with different levels of social complexity and estimated their recombination rates based on population linkage disequilibrium using the software packages LDhat and LDhelmet. The estimated recombination rates do not deviate from typical rates of non-social insects and are clearly lower than rates found in eusocial Hymenoptera. This shows that sociality is not always associated with elevated recombination and that an alternative explanation for the elevated rates

in eusocial Hymenoptera might be necessary. We hypothesize that the high recombination rates in eusocial hymenopteran insects are instead related to their skewed sex ratios of reproductive individuals. In hymenopteran colonies, there is usually a high number of haploid males that compete for mating with a single female, whereas termites typically have an equal number of reproductive males and reproductive females. In hymenopteran colonies, the haploid males can be seen as an efficient system for testing different haplotypes, as both dominant and recessive variants are expressed and unsuccessful individuals are lost without any cost for the colonies.

In this study, we have also analysed the distribution of recombination events across the termite genome. Many vertebrate genomes have recombination hotspots, which are 1-2 kbp regions of strongly elevated recombination rate. Hotspots have also been found in a butterfly species but not in most other insects studied so far. We have found strong evidence of hotspots in the termite genome, with 50% of the recombination events localized to <0.5% of the genome for both of the studied species. Both species also have full-length copies of the gene for the zinc finger protein PRDM9, which controls hotspot locations in many vertebrates. It is still unclear whether PRDM9 is active and has the same function in termites, but if it does, this could indicate a more ancient role of this protein than previously known.



Linkage disequilibrium based inference method reveals stable recombination hotspots in the house sparrow

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POSTER SESSION 3 - P03.006, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Meiotic recombination is a fundamental mechanistic feature of sex and an important evolutionary force. Yet, recombination rates and landscapes vary from species to chromosome level. Understanding the causes and consequences of this variation remains a major challenge. The PRDM9 protein has been identified as a major recombination modifier in several vertebrates, particularly mammals. It leads to the rapid turnover of recombination hotspots between populations. This protein is absent in many taxa, including birds, where recombination landscapes are evolutionarily stable. Without the confounding effect of PRDM9-driven hotspot turnover, this makes the avian family a relevant taxonomic group to investigate another important level of recombination variation: differences between male and female recombination rates, known as heterochiasmy. Among birds, the house sparrow (*Passer domesticus*) is an emerging model system for studying the causes and

consequences of recombination. We used linkage disequilibrium from whole-genome polymorphism data to investigate the fine-scale population recombination landscape in three wild Eurasian house sparrow populations. The degree of LD between variants reflects the variation in recombination rates along the genome, a key step in uncovering the genetic and mechanistic drivers of recombination. Analysis of these LD-based maps confirms the presence of stable recombination hotspots between the three sparrow populations, mainly located near promoter regions and CpG islands. With the forthcoming integration of pedigree data and single cell sequencing, I aim to identify the functional genomic features associated with recombination events, to distinguish between male and female recombination hotspots and to present first insights into the genetic basis of sex differences in recombination rates and hotspot usage in the house sparrow.



Detecting genomic incompatibilities and barriers during divergence with gene flow in *Formica* ants

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MEETING ROOM 129+130, August 20, 2025, 10:30 AM - 12:30 PM

Two current goals of speciation research are to understand whether genomic barriers to gene flow persist after contact between divergent lineages, and to identify the loci underlying such reproductive barriers. Locating barrier loci in empirical data is difficult due to the necessity of understanding the often complex demographic history of focal taxa and the heterogeneity in evolutionary forces across the genome. Furthermore, when identified, barrier loci are usually studied in isolation of one another, yet theoretical models predict an important role for coupling or epistasis in barrier persistence. In one classical model of epistasis, the Bateson-Dobzhansky-Muller (BDMI) model, incompatibilities are thought to be easily eroded by gene flow. However, this is rarely tested. Here we show empirically that BDMIs are persistent barriers when speciation happens with gene flow. We take advantage of the *Formica* hybrid wood ant system and recent methodological developments to (i) map barrier loci showing reduced migration during divergence between two species with the demographically explicit scan gIMble. This analysis used four whole-genome sequences from each parental species (*Formica aquilonia* and *F. polyctena*) and (ii) identify candidate BDMIs in naturally occurring hybrids through an imbalanced recombinant haplotype frequency analysis using the X(2) statistic. The X(2) method is related to linkage disequilibrium methods in that it looks for non-random associations between alleles, specifically missing haplotype combinations at loci of known ancestry. Here we used 386 whole-genome sequences from a single hybrid population in Finland. We find that a large

number of both barriers and BDMIs are scattered across the genome, rather than a small number of clusters. Furthermore, candidate BDMIs partly overlap with the long-term barrier regions identified by gIMble. Intriguingly, candidate BDMIs show interconnectivity, and the number of pairwise interactions a BDMI has impacts how effectively it reduces gene flow: BDMIs with many pairwise interactions to other BDMIs tend to reduce gene flow more effectively than those with fewer interactions. Finally in regards to function, long-term barriers identified by gIMble arise outside regions of both gene coding sequences (CDS) and transposable elements. In contrast, regions where long-term barriers and BDMIs co-locate are significantly associated with introns. Our results draw attention to an underappreciated aspect of incompatibilities: the structure of the network in which they interact, which has evolutionary consequences. Furthermore, it suggests persistent BDMI loci may have specific molecular characteristics, for example acting as hub genes in molecular pathways. The overlap between candidate BDMIs and regions of no gene flow between species indicates that incompatibilities have persisted despite divergence with gene flow between the wood ant species. Finding that long-term BDMIs are significantly associated with intronic regions implies a potential role of alternative splicing or gene regulation in incompatibilities, rather than CDS divergence. Overall, our results highlight the underappreciated impact of multilocus BDMIs and the need to consider network connectivity of BDMIs in future work.



Genomic incompatibilities and admixture between oviparous and viviparous lizards at a hybrid zone

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MEETING ROOM 129+130, August 20, 2025, 10:30 AM - 12:30 PM

Live-bearing young (viviparity) or to egg-laying young (oviparity) is one of the most fundamental alternative life history traits found in animals. Viviparity has arisen from oviparity many times in squamates, yet instances where interbreeding is found between different parity modes are exceedingly rare. Hybridisation between individuals of different parity modes must have also occurred early in the origins of viviparity, thus, hybridization between lineages of alternative parity modes can provide unparalleled insight to the genetic mechanisms of this evolutionary innovation. We studied a unique hybrid zone between oviparous and viviparous Eurasian common lizards (*Zootoca vivipara*) to examine the extent to which genomic incompatibilities inhibit hybridisation, their arrangement and linkage across the genome, and the fine-scale spatial patterns of their transition between parity modes. To do so we deeply evaluated the genome-wide variation within and among individuals using whole genome resequencing of 266 lizards. We found very

high levels of admixture between oviparous and viviparous genomic backgrounds across the full continuum of possibilities, including a relatively large proportion of individuals with intermediate ancestry proportions. Gene flow is symmetrical into both parental lineages. We found a very narrow cline width of 3 km between the parity mode lineages. Steepness of the cline differed between chromosomes and genomic cline outliers were found on all autosomes. Leveraging a previously generated high resolution linkage map for this species, we plotted recombination rate across the genome and found genomic regions of excess ancestry (viviparous and oviparous combined) did not differ from background. Overall, our findings suggest that hybridisation between these parity mode lineages is long-standing and isolating mechanisms (or lack thereof) are such that genomic incompatibilities are rare. These findings suggest that the evolution of viviparity involves gradual and genome-wide divergence.



Inferring the evolutionary history of *Caenorhabditis elegans* while accounting for its self-fertilising reproductive mode

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MEETING ROOM 129+130, August 20, 2025, 10:30 AM - 12:30 PM

Self-fertilisation extensively influences the evolutionary history of hermaphroditic species, by reducing effective population size and effective recombination rate, and increasing linkage disequilibrium (LD). These effects are usually ignored in population genetic inference methods, which overwhelmingly assume outcrossing. Here, we investigated how strengthened LD affects the population structure analyses and demographic inferences in *Caenorhabditis elegans*, a predominantly self-fertilising nematode. We applied a recently-published method for jointly inferring the demographic history and the self-fertilising rate of different *C. elegans* population. Population structure analysis suggests that strains from different Hawaiian islands are more genetically divergent than strains outside Hawaii, with strains from Kauai, the most ancient islands

of Hawaii, exhibiting the highest level of intrapopulation divergence. Pruning variants based on pairwise LD reduced the genetic divergence among Kauai strains but had little effect on the general pattern of population structure. For the Big Island population from Hawaii, the demographic inference with self-fertilisation considered estimated a selfing rate of 99% and a population decline followed by a massive increase. Further investigations comparing methods with or without self-fertilization taken into account and on how extensive linked selection may bias the demographic inference will provide important information on how to best infer the evolutionary history of populations with unusual reproductive mode and/or strong LD caused by uniparental reproduction.



Integrating LD- and Coalescent-Based Approaches Reveal Stability-Driven Genetic Diversity in the Adaptive Radiation of Lake Tanganyika Cichlids

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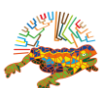
MEETING ROOM 129+130, August 20, 2025, 10:30 AM - 12:30 PM

Fluctuations in effective population size (N_e) leave detectable signatures in the genome, particularly in linkage disequilibrium (LD) patterns across loci, offering a powerful tool to reconstruct demographic history in natural populations. Neutral theory suggests that larger, stable populations maintain greater genetic diversity due to reduced genetic drift. However, the relationship between population size and genetic diversity remains debated, especially in natural populations with variable demographic histories and selective pressures. Here, we apply and compare both, LD-based two-locus and coalescence-based approaches to explore how historical population dynamics have shaped the present-day genomic diversity in the adaptive radiation of cichlid fishes in Lake Tanganyika, the ecologically, morphologically and behaviorally most diverse cichlid assemblage in Africa.

Using whole-genome data from 620 individuals across 40 species, we capture deep-time dynamics through historical mutation patterns, while LD-based inference enables fine-scale resolution of recent demographic changes by leveraging allele frequencies correlations. Our LD analyses revealed that current N_e is strongly associated with ecological traits —

specifically habitat depth and trophic level, possibly reflecting ongoing ecological processes. Cluster analyses revealed five demographic groups, all of which show a strong population decline during Lake Tanganyika's largest water level drop (500–600 m below present), likely fragmenting the lake and its inhabitants into sub-basins. A positive correlation between genetic diversity and coalescent-based N_e stability supports the 'stability hypothesis', suggesting that species less affected by N_e fluctuations, such as bottlenecks, maintain higher genetic diversity. In contrast, LD-based recent estimates showed no such pattern and were more closely associated with ecological traits. In this scenario, ecological habitat choice, crucial to current connectivity, also influenced historical demographic patterns and diversity, with deep-water species showing higher current genetic diversity and greater susceptibility to population fluctuations from past lake-level shifts.

Our research underscores the importance of integrating and discussing multiple methods for investigating demography and elucidates the intricate mechanisms driving cichlid population dynamics with a critical role of the ecological context in adaptive radiation.



Utilizing linkage disequilibrium (LD) to improve signal to noise ratios in outlier analyses

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MEETING ROOM 129+130, August 20, 2025, 10:30 AM - 12:30 PM

Identifying genomic regions associated with fitness in natural populations is central to understanding and predicting how organisms evolve in a changing environment. This can be achieved by identifying outlier loci in empirical distributions of population genetic statistics such as polymorphism and divergence, through associations between genotypes and environmental variables or through allelic frequency changes associated with survival in artificial selection experiments. However, when genetic structuring is high, based on statistical association alone it is impossible to distinguish a locus that has high allele frequency difference between environments/experimental treatments due to selection from a locus where this is due to random genetic drift. This results in either high false positive rates or low statistical power. A second major challenge comes from the failure to account for non-independence between loci making corrections for multiple testing in high density SNP data unnecessarily conservative. Linkage disequilibrium network analysis (LDna) uses pairwise estimates of LD to partition correlated sets of loci into LD-clusters representing distinct phylogenetic signals in the data. The LD-clusters can for instance be associated with population structuring, chromosomal rearrangements, sex-determination and not the least selection. Recent studies have demonstrated that LD-network-based complexity reduction in combination with association analyses may offer a solution to

both challenges in outlier analyses. This is because selection is known to leave strong LD-signature in population genomic data, such that LDna can both reduce the number tests (complexity reduction) while simultaneously focusing on loci from that are the most likely to be involved in selection. However, LDna is impractical for large data sets because it relies on pairwise LD estimates. Furthermore, the underlying premise that SNPs close to selected loci are overrepresented among LD-clusters have not been explicitly tested in simulations. However, from simulated data we discovered that directly factoring the LD-structure surrounding individual SNPs in the test statistics from association analyses could improve the ability to predict both the association with and the physical distance from selected loci, circumventing the need for network-analyses. This method i) reduced false positive rates in nearly all simulated data sets, ii) allowed for the detection of outlier loci that are physically closer to selected loci and iii) when applied to empirical data from the three-spined sticklebacks, identified multiple outlier regions not found in previous LDna based analyses using only a fraction of the computational time. However, we also demonstrate that LDna can still play a central role in partitioning the significant SNPs into outlier regions as well as in understanding the correlation structure among them for instance caused by genome wide epistatic interactions.





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A comprehensive representation of selection at loci with multiple alleles that allows complex forms of genotypic fitness

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*POSTER SESSION 1 - **P01.023**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM*

Genetic diversity is central to the process of evolution. Both natural selection and random genetic drift are influenced by the level of genetic diversity of a population; selection acts on diversity while drift samples from it. At a given locus in a diploid population, each individual carries only two alleles, but the population as a whole can possess a much larger number of alleles, with the upper limit constrained by twice the population size. This allows for many possible types of homozygotes and heterozygotes. Moreover, there are biologically important loci, for example those related to the MHC complex, the ABO blood types, and cystic fibrosis, that exhibit a large number of alleles. Despite this, much of population genetic theory, and data analysis, are limited to considering biallelic loci. However, to the present, what is lacking is a

flexible expression for the force of selection that allows an arbitrary number of alleles (and hence an arbitrary number of heterozygotes), along with a variety of forms of fitness. In this work, we remedy this absence by giving an analytical representation of the force of selection that emphasises the very different roles played by the diversity of the population, and the fitnesses of different genotypes. The result presented facilitates our understanding and applies in a variety of different situations involving multiple alleles. This includes situations where fitnesses are: additive, multiplicative, randomly fluctuating, frequency-dependent, and it allows fitnesses which involve explicit gene interactions, such as heterozygote advantage.



A Simulation Study Utilising REvoSim to Investigate the Impact of Predation on Species Diversity Across Environmental Heterogeneity

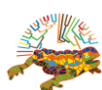
Peacock C¹

¹University Of Manchester

POSTER SESSION 1 - P01.032, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Predation plays a pivotal role in shaping biodiversity patterns, particularly across environmental heterogeneity gradients, such as the latitudinal biodiversity gradient. This research replicates and expands upon the findings of Freestone et al. (2011), which demonstrated the increased negative impact of predation on species richness in equatorial environments. Utilising the software REvoSim, a spatially explicit eco-evolutionary simulation platform, we conducted 1,100 runs across a range of environmental heterogeneities (S1-S10, and S15) under two predation conditions (absent and evolving predators). These simulations provided data on species richness, population dynamics, and community structure under the various environmental and predator conditions. Our findings support the conclusions made by Freestone et al., showing that predation has a more significant impact on species richness at intermediate to high heterogeneity levels. However, our results show that species

richness plateaued as heterogeneity increased, rather than scaling linearly. The presence of evolving predators led to a reduction in species count, reduced maximum species population sizes, and a lower number of total organisms within the system. Conversely, measures of species evenness were found to be higher with predators present, indicating that predation helps balance community structure by limiting species dominance. These findings suggest that the relationship between species richness and environmental heterogeneity is constrained by underlying factors such as resource availability and carrying capacity, which in turn limit the negative effect of predation at the higher environmental heterogeneities. This research has significant implications to the fields of evolutionary ecology and conservation for maintaining biodiversity in more diverse environments, particularly in equatorial ecosystems.



An interpretable machine learning-based alternative to genome-wide association studies (GWAS), and its application in a wild population

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POSTER SESSION 1 - P01.034, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: The identification of genetic markers associated with phenotypic traits remains a key objective in evolutionary biology as well as in medical research and animal and plant breeding, but studies in wild populations are still relatively rare. Traditional genome-wide association studies (GWAS) have known limitations, particularly for the analysis of complex traits. In addition, an often under-evaluated downside of GWAS is that they are exclusively based on p-values, which combine effect size and uncertainty and are thus unreliable measures of variable importance.

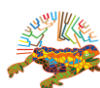
Materials and Methods: Here, we investigated alternative measures of variable importance for identifying associations between Single Nucleotide Polymorphisms (SNPs) and phenotypic traits. In particular, we used so-called “Shapley values”, which originally were constructed for simple regression models to capture the contribution of each individual variable to the amount of variance explained by the total model. Since SNP data typically involve far more markers (p) than data points (N), standard regression models fail. We therefore employed machine learning models including boosted regression trees (XGBoost) and ridge regression, both of which enable estimation of variable importance using Shapley Additive Explanations, or SHAP values.

The approach was tested on genomic data comprising approximately 70,000 SNP markers and phenotypic measurements of 4000 wild house sparrows (*Passer domesticus*) from an insular metapopulation off the Helgeland coast in northern Norway. The analysis was performed for three traits: body mass, tarsus length and wing length. As a comparison, we performed a classical GWAS using linear mixed models. In both approaches we accounted for population

structure. To assess and compare the quality of SNP-trait associations identified by GWAS and SHAP, we also simulated phenotypes based on additive genetic models for a range of genetic architectures, and performed GWAS and SHAP analyses on the simulated traits.

Results: SHAP results give complementary information to GWAS, with the two methods showing little overlap in the SNPs identified as important. GWAS identified no SNPs surpassing the strict Bonferroni-corrected significance threshold for the three highly polygenic traits in the house sparrow system, illustrating its limited power under genetic architectures and sample sizes common for studies of wild populations. This result is consistent with the result from our simulation studies, where GWAS and SHAP were unable to identify associated regions for highly polygenic traits. On the other hand, both GWAS and SHAP effectively identified SNPs linked to traits simulated under more oligogenic architectures. In addition, our analyses illustrate that SHAP values contain information about the genetic architecture of traits in a way p-values are not designed for. In particular, SHAP values estimate the phenotypic variance explained per SNP, which enable inference about how many SNPs contribute to different levels of phenotypic variance.

Conclusions: Motivated by the intrinsic limitations of p-values, we have suggested alternatives to GWAS for mapping genes underlying variation in phenotypic traits. Our findings suggest that importance measures based on SHAP that we here tested for boosted regression trees, as well as a linear model based on shrinkage, may offer a promising alternative for genomic inference compared to GWAS.



Comparison of classical and AI-based computational tools for predicting deleterious mutations in plants

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POSTER SESSION 1 - P01.046, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Understanding the role of negative selection and the impact of unfavourable mutations on adaptation has become increasingly relevant in Evolutionary Biology. Deleterious mutations can accumulate as genetic load, reducing fitness and threatening the viability of organisms. Current approaches use bioinformatic methods for predicting mutational deleteriousness in order to estimate genetic load from genomic data. Conventional approaches estimate the effect of mutations based either on the evolutionary conservation of the mutated site or on the predicted impact on protein structure and function. Recently, artificial intelligence (AI) techniques have emerged as powerful tools for predicting mutational effects, offering new opportunities to refine genetic load estimates. However, the performance of predicting tools remains poorly explored in plants, with empirical studies mostly limited to cultivated and model species. In addition, most AI-based deleteriousness prediction methods are focused on human variants.

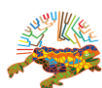
To address this gap, we performed a comparative evaluation of seven methods for predicting deleterious genetic variants in plants. First, we assessed three classical tools: SIFT, PROVEAN and SNPEff. Second, we included a supervised, plant-specific machine learning model: PPVED. Third, we evaluated ESM-1v, an unsupervised deep learning protein language model (PLM) trained on 98 million protein sequences, which has recently emerged as one of the top-performing models for predicting mutational effects. PLMs stand out for their ability to learn complex contextual patterns, and evolutionary and functional constraints underpinning protein sequence data via unsupervised training. This

allows capturing residue dependencies, structural relationships, and biologically meaningful signals without relying on labelled examples. The final two approaches align with current state-of-the-art practices and involve leveraging the internal sequence representations (embeddings) extracted from PLMs through two distinct strategies: (1) by using these embeddings as input features to train supervised models specifically for predicting the mutational effects of plant variants; and (2) extending this approach by combining PLM-derived features with scores from classical conservation-based methods, such as SIFT.

This evaluation was conducted using a curated dataset of 12,865 SNPs, functionally annotated as neutral or deleterious, in 6,172 protein sequences from 433 plant species, retrieved from UniProt.

Preliminary results indicate that PLM-based approaches, particularly those using mean embeddings as input features, outperformed classical tools in predicting mutational effects on the curated plant dataset. Moreover, combining PLM-derived features with SIFT scores consistently improved predictive performance across all strategies.

As a follow-up of this study, we will apply these novel methods to tree population genomic datasets, aiming to estimate genetic load and investigate its impact on plant phenotypic traits, an area that remains largely unexplored. This work contributes to evaluating the potential of AI methods for deleteriousness prediction and introduces a new framework for estimating genetic load from genomic data.



Crossovers and Consequences: Genomic Prediction Reveals Selection on Recombination Rates in a Natural Population

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POSTER SESSION 1 - P01.027, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Meiotic recombination during gamete formation is a fundamental evolutionary process. It is required to ensure proper chromosome segregation and promotes genetic diversity by shuffling alleles into novel haplotypes. This can create beneficial allele combinations and break up linkage between beneficial and harmful alleles, thereby facilitating adaptive evolution. However, high recombination rates also carry risks, such as introducing mutations at crossover sites, and potentially disrupting favorable allele combinations. Thus, recombination rates could affect individual fitness in opposite directions, suggesting a complex relationship with individual fitness.

Investigating the impact of recombination rates on fitness is complicated by the fact that identifying crossover positions during meiosis - and thus finding the crossover count - is only possible for individuals with genotyped offspring. This introduces a sampling bias which prevents measuring the recombination rates of individuals that are reproductively unsuccessful, excluding them from potential fitness analyses. Failure to account for these unmeasured individuals could potentially bias analyses of the fitness effects of recombination rates.

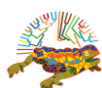
Materials and methods: We used a long-term house sparrow study system from northern Norway with extensive pedigree, genotype, and fitness data to investigate the relationship between individual recombination rates and fitness components. The genomic and pedigree data were leveraged to measure autosomal crossover count (ACC) in adult house sparrows, resulting in one ACC measure per genotyped parent-offspring pair. We estimated sex-specific individual breeding values for ACC by using the phenotypic ACC measures for each parent as responses in a genomic prediction model. The genomic prediction, implemented as a Bayesian G-BLUP model, allowed us to estimate these

breeding values in more than 2,600 phenotyped individuals, as well as in more than 2,900 additional individuals with no recorded genotyped offspring, correcting the aforementioned sampling bias and increasing the sample size of the subsequent fitness analyses.

We then related the estimated breeding values to sex-specific fitness components, including annual reproductive success and annual survival. As the individuals without ACC measurements had higher uncertainty in their estimated breeding values, we used measurement error modelling to account for the heterogeneous breeding value uncertainties.

Results: We found that individual ACC was related to male and female annual survival, with predicted annual survival peaking at intermediate breeding values for individual ACC. We also found weak evidence that males with intermediate breeding values had higher annual recruit production, but no evidence of a relationship with reproductive success in females. Altogether these results suggest potential stabilizing selection against extreme values of ACC, especially in male house sparrows.

Conclusions: We demonstrate that individual variation in recombination rates influences fitness in a wild house sparrow population, with evidence of stabilizing selection on ACC particularly in male house sparrows. To interpret this association, we consider possible underlying biological mechanisms. On the methodological side, we present genomic prediction as a tool to address sampling biases. This novel approach allowed us to investigate the fitness consequences of a trait that can only be directly measured in reproductively successful individuals and allowed us to include several thousand individuals in the analysis that would otherwise have been excluded.



Detection of eco-evolutionary dynamics in communities using Joint Species Distribution Models

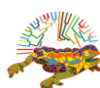
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¹University Of Konstanz, ²Université de Franche-Comté

POSTER SESSION 1 - **P01.014**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Biodiversity at the metacommunity scale is typically influenced by a number of environmental, spatial, biotic, and stochastic factors. At the same time, these factors impact the evolution of individual species, as sites present different local selection pressures and connectivity can impact gene flow and genetic drift. Identifying the relative impacts of environmental, spatial, biotic, and other drivers on community composition across spatial and temporal scales has been greatly facilitated by joint species distribution models, but these models have yet to consider the impact of microevolution on community composition. We used Hierarchical Models of Species Communities (HMSC) to analyze simulated data of populations and communities, including in a large evolving metacommunity

model, to establish whether HMSC can sufficiently quantify the contribution of phenotypic evolution for metacommunity composition. The models successfully partitioned variance contributed by environmental, spatial, and evolving phenotypic drivers, and also estimated site- and time-specific covariance. We also applied the HMSC with trait evolution model to an existing dataset studying trait change and community dynamics in an experimental aquatic plant system. With this approach, we demonstrated that JSDM models such as HMSC can be used to estimate effect sizes of trait evolution for community composition, and thus can reflect the numerous complex, interacting processes of the eco-evolutionary dynamics that operate in many natural systems.



Division of Labour in the Evolution of Group Exclusion

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POSTER SESSION 1 - **P01.045**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: During Major Evolutionary Transitions, the evolutionary interests of smaller units become aligned. This often occurs through high relatedness. Highly related groups can be formed either via low dispersal or via group exclusion of unrelated immigrants, but the latter has received less theoretical attention. I thus explore whether division of labour can facilitate the evolution of group exclusion.

Materials and methods: I built an infinite island model with dominance hierarchy. The dominant individual in a group produces $c(1-x)$ offspring, and each subordinate $p(1-x)$ offspring, where p determines within-group skew, and x is the exclusion effort. Then the offspring disperse with probability d , with a fraction k of dispersers dying in transit. Surviving dispersers successfully gain entry to a random patch with probability determined by local adults' exclusion effort. Adults then die with probability $1-(1-g)(1-x)$, where g is the baseline mortality rate, and x the exclusion effort. If a dominant dies and some subordinates survive, then a random surviving subordinate is selected to become the new dominant. The vacancies left by dead adults are then filled up by offspring drawn at random from the local offspring pool (including both native born and immigrant young). Those offspring that fail to obtain a breeding spot then die. Using the

model described above, I built equations that capture the fitness of each individual given their own traits, local trait and population trait. I used the direct fitness approach (a.k.a. the neighbour-modulated fitness approach) to include the effect of kin selection. I used an adaptive dynamic approach to find the direction of selection which was then used to determine equilibria.

Results: Evolutionary stable states (ESS) are characterized as open or closed depending on whether exclusion evolves.

Allowing for Dominance Hierarchy (even without skew) increases the parameter range over which closed ESS occur. The greater stability of closed ESS is a result of division of labour – in the parameter space where models without dominance hierarchy cannot form stable closed ESS, the model with dominance hierarchy yields outcomes in which some individuals breed while others work to exclude newcomers, analogous to the soma / germ divide.

Conclusions: Group exclusion is a common, spiteful action observed in nature, yet its theoretical implication is rather understudied. The results show that from simple assumptions, it is possible to generate patterns observed in nature. This suggests further studies into the consequence of group exclusion may be fruitful.



Estimating bacterial mutation rates with simulation-based methods (ABC)

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POSTER SESSION 1 - **P01.021**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The mutation rate is a key parameter of evolution as it defines the speed at which genetic diversity is generated de novo. It is defined as the number of mutation occurring per base pair per cell division (equal to generation for asexual organisms). A traditional approach to estimate mutation rate for bacteria is the fluctuation test, which consists in growing bacteria in a non-selective medium for then plating them in a selective environment in order to reveal the number of mutants (survivors) carrying a resistance to that stress. A mathematical model is then fitted on these experimental data to infer mutation rate. However, model makes restrictive assumptions regarding the effect of the mutations on the fitness (no effect) and the growth conditions (no death in the culture) which probably do not represent reality.

The objective of our work is to develop a new simulation-based computational method for the inference of mutation rate from experimental data (fluctuation tests), taking into account non-standard demographics (death) and fitness effect of the mutation.

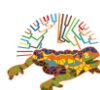
Our approach relies on Approximate Bayesian Computations: it consists in using a simulator combined with MCMC algorithms (Metropolis-Hastings) to find the parameters which best explain the experimental data.

We tested it on two types of tasks:

- estimation of 1 parameter : mutation rate, when other parameters (death rate and fitness effect) are known
- simultaneous estimation of 2 parameters : mutation rate and fitness effect or mutation rate and death rate.

As today, this method already provides encouraging results. It competes well with the reference tools (rSalvador, which uses the Ma-Sandri-Sarkar maximum likelihood estimator, and Flan's probability generating function) in simple scenarios where these tools work.

Due to its simulation-based nature, it can also generalize to more complex scenarios where these reference methods do not work.



EvAM-Tools: a tool for Evolutionary Accumulation Models

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POSTER SESSION 1 - P01.022, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Cancer progression models (CPMs) and evolutionary accumulation models (EvAMs) were developed to discover dependencies in the irreversible acquisition of binary traits from cross-sectional data. They have been used in computational oncology and virology but also in widely different problems such as malaria progression and antimicrobial resistance. These methods have been applied to predict future states of the system, identify evolutionary routes of feature acquisition, and improve patient stratification, and new methods continue to be developed. Despite their existing applications and their promise for evolutionary-based treatments and therapeutic target choice, these methods are not widely used. A possible reason is the difficulty, for end users, to have easy access to experiment with the different methods to understand their assumptions and implications, as well as the lack of access to the existing state-of-the-art implementations. For developers, the difficulty of comparing and benchmarking existing and new approaches is also a likely hindrance.

Results: EvAM-Tools is an R package and Shiny (<https://shiny.rstudio.com/>) web app, also available as Docker images, that provides a unified interface to state-of-the-art EvAMs. It allows users to:

- Run state-of-the-art EvAMs, including Conjunctive Bayesian Networks (CBN ---and their MC-CBN version---), Oncogenetic trees (OT), Mutual Hazard Networks (MHN), Hidden Extended Suppes-Bayes Causal Networks (H-ESBCNs ---PMCE---), and Disjunctive Bayesian Networks (DBN, from the OncoBN package), among others that we continue incorporating, with a single function call.
- From the fitted models, represent, graphically, the fitted models (DAGs of restrictions or matrix of hazards, as appropriate), the transition matrices and transition rate matrices (where

appropriate) between genotypes and show frequencies of genotypes sampled from the fitted models.

- Generate random EvAMs or CPMs.
- Using the shiny app, easily visualize the effects of changes in genotype composition on the fitted models by entering user-defined cross-sectional data using a GUI.

The GUI provided via the web interface allows fast construction, manipulation, and exploration of EvAM models, making it easy to gain an intuitive understanding of what these methods infer from different data sets as well as what kind of data are to be expected under these models.

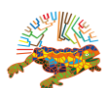
Users can compare results from different methods/models, as well as experiment and understand the consequences of changes in the input data on the returned inferences. This also allows understanding how a given method performs when data have been generated under another (or its own) model.

EvAM-Tools includes extensive documentation, in the form of standard R help, clickable help and tooltips in the web application, and detailed tutorials of use.

Availability:

- Source code:
<https://github.com/rdiaz02/EvAM-Tools>.
- Publicly and freely accessible web app:
<https://www.iib.uam.es/evamtools/>
- Docker images:
<https://hub.docker.com/r/rdiaz02/evamshiny>
and
<https://hub.docker.com/r/rdiaz02/evamrstudio>

Conclusions: EvAM-Tools facilitates broader adoption of EvAMs by providing a user-friendly interface and fostering comparison of methods. This will lead to a better understanding and increased use of these methods and will encourage further methodological work and their extension to other scenarios. EvAM-Tools is thus a valuable tool for computational oncologists and more broadly to evolutionary biologists.



Evidence that human skill level is more reactive to developmental history than to genes or environment

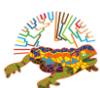
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POSTER SESSION 1 - P01.028, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The human brain is influenced by genes, environment, and developmental history. Extensive efforts seek to identify the causes of observed variation in behaviour and disease. Moreover, it is of primary interest to predict the brain's response to influences, both at present and over our evolutionary history. Here we use a mathematical model that mechanistically recovers major aspects of human brain development and evolution to predict the response of adult skill level to point influences earlier in development. We show that adult skill level is much more reactive to changes in developmental history than to genetic, environmental, or social changes over life during the recovered evolutionary trajectory from *H. habilis* to *H.*

sapiens. This occurs despite our finding that adult skill level has evolved an increased reactivity to environmental and social change, and a decreased reactivity to genetic change, which is consistent with observation. We further show that adverse genetic and environmental conditions that would developmentally yield low adult skill level can be more than compensated by time-specific changes in the environment or by slight changes in developmental history, yielding outstanding adult skill level. Our analyses identify developmental history as having a major ability to influence adult human skill level.



GHIST: the Genomic History Inference Strategies Tournament

Struck T¹, Vaughn A², Daigle A³, Ray D³, Noskova E⁴, Sequeira J⁵, Antonets S⁶, Alekseevskaya E⁷, Grigoreva E⁸, Raines E⁷, McMaster E⁹, Kovacs T⁹, Ragsdale A¹⁰, Moreno-Estrada A¹¹, Lotterhos K¹², Ralph P¹³, Siepel A¹⁴, **Gutenkunst R¹**

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*POSTER SESSION 1 - **P01.017**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM*

Evaluating population genetic inference methods is challenging due to the complexity of evolutionary histories, potential model misspecification, and unconscious biases in self-assessment. We present the Genomic History Inference Strategies Tournament (GHIST), a community-driven competition designed to independently evaluate methods for inferring evolutionary history from population genomic data. The inaugural GHIST competition featured four demographic history inference challenges of increasing complexity: a bottleneck model, a split with isolation model, a secondary contact model with demographic complexity, and an admixture model. Data were provided as error-free VCF files, and participants submitted numerical parameter estimates that were evaluated using relative root mean squared error. Approximately 60 participants competed over a five-month period, using diverse approaches. Results

revealed the current dominance of site frequency spectrum-based methods for simpler scenarios, while highlighting the advantages of flexible model-building approaches like GADMA for complex demographic histories. We discuss insights regarding evaluation strategies, including the challenge of "metagaming" behavior, and outline plans for future iterations with expanded challenge diversity, including identification of adaptive selection. GHIST aims to drive methodological innovation through transparent evaluation and community engagement, following successful models like CASP in protein structure prediction. By providing standardized benchmarks and highlighting areas for improvement, GHIST represents a significant step toward more reliable inference of evolutionary history from genomic data.



High levels of mitotic gene conversion are needed to effectively purge deleterious mutations in asexual organisms

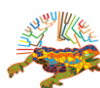
Kopčák D^{1,2,3}, **Hartfield M**¹

¹The University Of Edinburgh, ²Institute of Science and Technology Austria, ³Okinawa Institute of Science and Technology Graduate University

*POSTER SESSION 1 - **P01.019**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM*

Self-fertilisation and asexual reproduction are both hypothesised to lead to long-term extinction due to inefficient selection acting against deleterious mutations. Self-fertilisation can counter these effects through creating homozygous genotypes and purging deleterious mutations. Although complete asexuality lacks meiotic gene exchange, mitotic gene conversion is a mechanism for creating homozygous regions that could limit deleterious mutation accumulation in a similar manner to self-fertilisation. Here, we compare mutation accumulation in self-fertilising and facultative sexual populations subject to mitotic gene conversion, and quantify the relative efficacy of purging caused by the latter. We first show analytically that this purging is most effective with high levels of asexuality and gene conversion, and when

deleterious mutations are recessive. We further show using simulations that, when mitotic gene conversion becomes sufficiently high in obligate asexuals, there is a sudden reduction in the mutation count and a jump in homozygosity, reflecting mutation purging. However, this mechanism is not necessarily as efficient at purging compared to high levels of self-fertilisation, and elevated rates of mitotic gene conversion seem to be needed compared to empirical estimates. Hence, while mitotic gene conversion can remove recessive deleterious mutations, self-fertilisation seems to be a more effective mechanism for doing so. This talk highlights how contrasting modelling approaches (analytics and simulations) can be jointly used to tackle complex evolutionary topics.



How does ecological stability affect evolution?

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POSTER SESSION 1 - P01.050, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Predator-prey interactions are prevalent in many systems and are a well-known example of how eco-evolutionary dynamics may affect species interactions. While there has been a growing understanding of the effects of species interactions on evolution, we still know very little about how evolutionary trajectories may be altered by system stability, i.e. by whether new trait combinations lead to stable coexistence or population extinction. In this study, we investigate how the evolution of predator or prey traits shapes the stability of a predator-prey system, using a Rosenzweig-MacArthur predator-prey model. Using this model, we

mapped system stability to a range of different trait combinations for predator and prey, which allowed us to generate ecological fitness landscapes that identify regions of stable coexistence and predict the likelihood of different evolutionary trajectories for predator and prey. We then validated these fitness landscapes using simulations and examined how evolutionary trajectories and outcomes change when ecological stability is considered. Our findings shed light on the role of ecological stability in shaping the tempo and mode of predator-prey evolution.



How Green Is the Grass? Using Remote Sensing to Track Forage for Red Deer

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¹University Of Edinburgh

POSTER SESSION 1 - P01.016, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

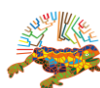
Remote sensing is a powerful tool for monitoring vegetation dynamics over time and space, but ecological applications depend on robust validation and biological interpretation. In this study, we use Normalised Difference Vegetation and Enhanced Vegetation indices (NDVI and EVI, respectively) derived from Landsat and MODIS satellite imagery to generate a high-resolution vegetation dataset for the Isle of Rum, Scotland – home to a long-studied population of free-ranging red deer (*Cervus elaphus*). Spanning the main growing season each year from 1990 to 2023, these data offer new opportunities to explore spatial and temporal trends in vegetation across Rum's heterogeneous landscape, which includes a mix of grassland (including deer forage), heath, and bog habitats.

A key strength of this system is the availability of long-term, fine-scale field data collected as part of the Isle of Rum red deer study (1972 – present). In particular, vegetation plots surveyed since the 1990s allow us to ground-truth satellite-derived greenness metrics and assess how well NDVI and EVI reflect biologically meaningful variation in plant productivity. Our analysis shows significant agreement between

remotely sensed and field-based vegetation trends, supporting the use of vegetation indices as proxies for plant growth in this system.

We also examine how vegetation dynamics vary across different habitat types within the Rum study area and demonstrate how satellite data can reveal spatial patterns that would be difficult or impossible to capture using field data alone. This spatial resolution adds a valuable new dimension to our ability to interpret environmental drivers of ecological processes, enabling future analyses that link fine-scale variation in vegetation to individual red deer life history traits — such as birth weight, overwinter survival and reproduction – and ultimately to population dynamics over time.

By integrating remote sensing data with field vegetation surveys, this work provides a case study in how to validate and apply satellite-derived vegetation indices in temperate systems. It offers a framework for using NDVI and EVI to assess spatial and temporal environmental variation in other ecological contexts where direct measurement is difficult or spatially limited.



Hues of blues: Using Comparative Genomics and Machine Learning to Uncover the Evolutionary Ecology of Color Vision in Krill

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POSTER SESSION 1 - **P01.035**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Do different species convergently evolve similar genetic adaptations and traits in response to shared environmental pressures? How can we use Machine Learning to advance evolutionary insights into trait evolution in non-model organisms? The present study employs a comparative analysis of krill opsin genes to explore these questions. Opsins are responsible for light sensitivity in the eyes of animals and are often adapted to the environment.

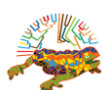
We recently assembled the 19 Gb genome of the Northern krill (*Meganyctiphanes norvegica*), a keystone macrozooplankton, and uncovered a greatly expanded opsin repertoire in this species compared to other crustaceans (Unneberg et al. 2024). A further analysis comparing Atlantic and Mediterranean populations of the species revealed evidence of adaptive divergence in allele frequencies across several opsins and numerous other genes involved in photoreception, circadian rhythm and photoperiodic regulation. These and similar findings in other marine organisms suggest that genetic adaptation to local ocean light conditions would commonly be critical in species with wide natural ranges and possibly involve convergent selection on homologous opsins and key amino acid tuning sites.

Materials and methods: Using transcriptomes from over 30 ecologically diverse krill species as models for parallel evolution and local opsin adaptation, we have identified more than 200 opsin genes. We next applied the novel OPTICS tool using machine learning models trained on the Visual Physiology Opsin Database (VPOD; Frazer et al. 2024) to predict the spectral sensitivity phenotype (λ_{max}) of each opsin. VPOD integrates experimental data from over a thousand wild-type or mutated animal opsins, including sequences and corresponding λ_{max} . OPTICS employs regression-based ML models, leveraging the DeepBreaks platform. It integrates

multiple regression algorithms, including Light Gradient Boosting Machine (LGBM), Random Forest (RF) and Extreme Gradient Boosting (XGB) to predict phenotypes from genetic sequences, while accounting for complex interactions like epistasis, and identifying critical amino acid tuning sites. These trained models show high predictive accuracy, detect known spectral tuning sites and mutation effects (including epistasis), and outperform traditional methods such as phylogenetic imputation in explanatory power, speed and error rates.

Results: We find that krill possess significantly more opsins than most other related crustaceans. ML predictions indicate a specific proliferation of middle-wavelength, blue-sensitive opsins, likely an adaptation for capturing dim light at depth, similar to deep-sea fish. Polar species express more diverse opsin repertoires than tropical species, suggesting that seasonal light variation influences opsin evolution across latitudes. We also analyze patterns of parallel and convergent spectral tuning and link predicted changes to gene duplication, selection and environment.

Conclusions: This study applies advanced ML tools to reveal novel insights into krill color vision evolution and the potential for repeatable molecular evolution among species. It highlights the benefits of combining systematic databases with ML for high-dimensional biological analysis, such as predicting molecular phenotypes of great importance to fitness and evolutionary ecology. While opsins are especially suited for such approaches due to their simple functional mechanisms, this framework is broadly applicable to other sensory proteins, such as predicting activation thresholds in thermosensory channels, offering a powerful tool for evolutionary biology and protein engineering.



Inference of dispersal and density parameters from spatial genomics data, using simulation-based machine learning methods

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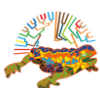
POSTER SESSION 1 - P01.044, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

During my PhD, I contribute to the development of indirect demographic inference methods. The aim is to provide the tools necessary to evaluate recent and local population history (mainly density and dispersal in the last dozens generations) using spatial and genomic data. This would inform conservation and management strategies while being less costly than approaches such as mark-recapture.

To achieve this goal, we use a simulation-based approach. Our method links the spatialised simulator GSpace (Virgoulay et al., 2021), the summary statistics calculating library GSumStat and the summary

likelihood statistical framework implemented in the infusion R package (Rousset et al., 2017).

GSpace implements both coalescent and forward time models of isolation by distance. GSumStat allows efficient computation of a wide variety of commonly used summary statistics. Infusion uses an iterative approach to infer joint distributions of likelihood of multiple parameters, by leveraging tools such as random forest machine-learning method and multivariate Gaussian mixture models.



Inferring the history of gene copy number evolution

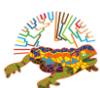
Otto M¹, **Wiehe T**

¹University Of Cologne

POSTER SESSION 1 - **P01.040**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Gene duplication plays a crucial role in the adaptive evolution and diversification of organisms by creating extra copies of genes that can evolve new functions while preserving the original. Duplicated genes can become fixed in populations or appear as copy number variants. However, inferring and dating these duplication events from present-day data is challenging, as gene copy count distributions could result from either a few ancient duplication events or many recent ones. Here, we introduce a novel model for inferring gene copy number evolution, which describes gene duplication and their evolution over time through a

random walk on a graph. We apply this model to the NB-ARC domain counts of NLR genes in *A.thaliana*, reconstructing the most likely 'duplication path' in the ancestral duplication graph to estimate duplication rates and date those gene duplication events. Using these estimates, we perform structured coalescent simulations that account for mutations and calculate key population genetic statistics, such as Tajima's D. Our findings indicate that naive sequence-based analyses of gene copy numbers may lead to misinterpretations of evolutionary history and underline the importance of including a duplication model.



Investigating the Maintenance of Alternative Reproductive Tactics in *Tetranychus urticae* males

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¹Groningen Institute for Evolutionary Life Sciences

POSTER SESSION 1 - P01.036, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

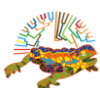
Background: Rapid environmental change necessitates predictive models of ecological and evolutionary responses, which can be effectively tested in species that display polymorphisms. Our project investigates the eco-evolutionary dynamics of male two-spotted spider mites (*Tetranychus urticae*), which exhibit alternative reproductive tactics (ARTs): fighter and sneaker tactics. Unlike other species where ARTs are linked to morphological differences, these tactics are behaviourally distinct yet morphologically identical.

Materials and Methods: To understand how these tactics are maintained and whether they represent fixed or flexible strategies, we employ advanced behaviour quantification tools to assess the behavioural phenotypes of individual males over time. High-resolution video recordings are used to capture male interactions under controlled conditions. Postural data are extracted using DeepLabCut, enabling individual

identification and tracking across extended behavioural observations.

Results: We successfully trained a mite posture detection model capable of accurately identifying and tracking individuals across varied lighting conditions and arena setups. This system provides a reliable foundation for future quantification of behavioural variation and tactic expression.

Conclusions: This work establishes the methodological groundwork for analysing the flexibility of alternative reproductive tactics in *T. urticae*. With individual identification in place, we are now positioned to test how ecological and intrinsic factors such as age, mating status, predation risk, and sex ratio may influence ART expression, contributing to a better understanding of behavioural plasticity and eco-evolutionary feedbacks in polymorphic mating systems.



Modelling the evolution of division of labour in clonal groups

Shillcock G¹, West S¹, Grafen A¹

¹Department of Biology

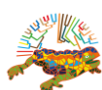
POSTER SESSION 1 - P01.051, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Division of labour, when cooperating individuals specialise in different tasks, occurs at every scale of biological organisation, from genes to complex animal societies. It is therefore crucial for us to understand the conditions which promote division of labour. Part of the complexity is that division of labour can confer fitness benefits for various reasons, in different biological cases. Previous theoretical research has often focused on only one of these reasons or specific types of tasks. There is a need for a theory that unifies different cases.

We model how a clonal group evolves to perform the various tasks contributing to its fitness. This framework is designed to be as general as possible by accommodating clonal groups of any size, performing any type and number of tasks. Our results apply to groups of genes, cells, organisms, or any

other evolutionary individuals. Our model shows there are at least three reasons why division of labour can be favoured by natural selection: (1) particular individuals may be predisposed to particular tasks, for example when individuals have differing access to resources; (2) focusing resources may increase individual efficiency, for example when task products do not mix well or tasks require up-front costs; and (3) specialists may contribute disproportionately to the group's aggregate performance, for example in cooperative defence. Group size is also shown to generally promote division of labour.

Our model allows us to categorise different forms of division of labour, according to certain biological scenarios. This can be used as a predictive tool for experimentalists to explain when division of labour does and doesn't occur.



Predator decision shape dynamics and stability of mimicry systems.

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POSTER SESSION 1 - P01.025, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Mimicry is a widespread anti-predator strategy where a prey species (the mimic) resembles an unprofitable species (the model) to deceive predators. Despite theoretical expectations for perfect mimicry, imperfect resemblances are common in nature. Here, we study the population dynamics and stability of both single- and multi-mimicry systems, focusing on the influence of varying predator recognition abilities. Using a theoretical framework that integrates optimal foraging theory, signal detection theory, abundance dependent recognition, and population dynamic, we demonstrate that intermediate mimic--model similarity stabilizes dynamics and promotes mimic abundance, thereby challenging past expectations for perfect mimicry. This stabilizing effect persists even when predators dynamically adjust their

recognition based on the mimic to model abundance ratio. Furthermore, this beneficial effect of imperfect mimicry persisted when we extended the framework to a multi-mimicry system involving both Batesian and Müllerian mimics. Batesian and Müllerian mimics confer different levels of protection to the system depending on the degrees of resemblance: Batesian mimics offer protection under intermediate similarity, while Müllerian mimics provide consistent protection under both intermediate or high similarity. Overall, our study offers novel insights into the prevalence of imperfect mimicry in nature --- it can represent an ecologically stable anti-predator strategy across a wide range of predator recognition abilities, and mimicry systems.



Quantifying camouflage evolution: digital phenotyping and macroevolutionary modelling in Neotropical butterflies

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POSTER SESSION 1 - P01.033, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

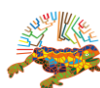
Recent advances in image analysis, morphological theory, and phylogenetic comparative methods are transforming how we study the evolution of complex traits in ecological contexts. These tools now enable us to quantify phenotypic complexity, decompose structural pattern components, and reconstruct the evolutionary sequences of traits across phylogenies.

Here, we apply these integrative methods to investigate the origin of complex cryptic patterns in butterflies and their interplay with behavioural innovation. We focus on lichen crypsis in Neotropical butterflies of the genus *Hamadryas*, which rest with wings open on lichen-covered bark, displaying intricate dorsal wing patterns that closely mimic the background's texture and colour. This open-wing resting posture—unusual for butterflies and more typical of moths—visually aligns the pattern with the background and likely enhances camouflage. This system provides an ideal context to assess how behavioural and morphological traits may have co-evolved in response to visually complex environments. Our study integrates three analytical components. First, we used high-resolution image analysis to quantify dorsal wing pattern complexity in 25 species of *Biblidinae*, employing edge-density metrics and spatial frequency analysis. We show that lichen-mimetic species exhibit significantly higher pattern complexity than their non-mimetic relatives. Second, we performed morphological decomposition based on the nymphalid ground plan to identify the

structural origins of this complexity. Lichen-mimetic species were found to modify conserved pattern elements and incorporate novel components, suggesting a layered evolutionary process. Third, we combined behavioural observations with phylogenetic comparative methods to reconstruct the evolutionary sequence of resting posture and pattern complexity. Our results indicate that the open-wing posture evolved prior to the emergence of complex lichen-like patterning, supporting a behaviour-first evolutionary pathway.

Together, these findings provide empirical evidence for the behaviour-first hypothesis—the idea that behavioural innovations can precede and facilitate morphological adaptation by reshaping the selective environment. In this case, a shift in resting posture appears to have enabled the exploitation of visually complex microhabitats, setting the stage for the subsequent evolution of cryptic wing patterning.

Our study demonstrates how the integration of digital phenotyping, morphological theory, and phylogenetic inference can test macroevolutionary hypotheses about trait interaction. It also exemplifies how classical natural history systems, such as butterfly mimicry, can be revisited using cutting-edge computational tools to uncover the dynamics of behavioural and morphological co-evolution. In doing so, we contribute to the broader effort of linking technological innovation with theory-driven questions in evolutionary ecology



Quantifying the effects of antibiotic resistance and within-host competition on strain fitness in *Streptococcus pneumoniae*

Krishna A¹, Tonkin-Hill G², Morel-Journel T³, Bentley S⁴, Turner P⁵, Blanquart F⁶, Lehtinen S^{1,7}

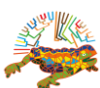
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POSTER SESSION 1 - P01.041, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

In multi-strain pathogens like *Streptococcus pneumoniae*, competition between strains shapes the dynamics of traits like virulence or antibiotic resistance. In pneumococcal epidemiology, this competition is traditionally conceptualised at the between-host scale - i.e. for hosts to colonise. However, because co-colonisation is common, strains also compete at the within-host scale. A growing body of theoretical work is highlighting the impact within-host competition has on dynamics at the between host scale. Yet, there is little empirical work on within-host competition. This poses a fundamental problem for our understanding of e.g. antibiotic resistance dynamics.

In this work, we address this problem using statistical analysis of the largest-to-date longitudinal dataset of pneumococcal carriage. We perform survival analyses on this dataset to quantify how within-host competition affects the rates of clearance

and establishment of pneumococcal strains. We find that the presence of a within-host competitor is associated with a 33% increase in clearance and a 54% reduction in establishment. Priority effects and serotype differences partially predict the outcomes of this within-host competition. Further, we quantify the effects of antibiotic resistance on between- and within-host components of fitness. Antibiotic consumption is associated with increased clearance rate for both susceptible and resistant strains, albeit to a higher extent in susceptible strains. In the absence of antibiotics, we find some evidence that resistance is associated with increased susceptibility to within-host competition, suggesting a fitness cost of resistance. Overall, our work provides quantitative insights into pneumococcal competition across scales and the role of this competition in shaping pneumococcal epidemiology.



Quantitative approaches reveal that viral cheats are highly abundant and form species-specific communities in natural infections of H5N1 avian influenza.

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POSTER SESSION 1 - **P01.038**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

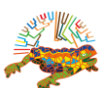
Viral infections are social processes. To successfully replicate inside infected cells, viruses must produce shared gene products, that can be used by multiple viral genomes within the same cell, and hence act as public goods. As a result, viral cheats can emerge, a type of molecular parasite formed by large deletions, which spread by exploiting public goods encoded by full-length viruses. Cheats exist across the viral universe, arise frequently in laboratory infections, and reflect the emergence of evolutionary conflict at the molecular level. If cheats are also common in natural viral infections, they could play a key role in viral population dynamics, pathogenesis, and adaptation.

Here, we report the widespread abundance of viral cheats across diverse species infected by the recently emerged strain of H5N1 avian influenza. Within thousands of newly available deep-sequencing datasets, we use bioinformatic tools to find that the majority of infected animals contain viral cheats at detectable frequencies. Strikingly, in more than one in four animal infections, cheats reach a relative abundance greater than 50%, indicating that they are the majority variant within that population.

We then interrogate this dataset using mathematical and statistical tools drawn from evolution and ecology. We ask three key questions. Firstly, we use statistical tools

from community ecology to show that viral cheats form distinct communities within each infected host, such that different host species are characterised by different viral cheat sequences becoming highly abundant. This indicates that separate mutational and selective forces shape the emergence and evolution of cheating across host species. Secondly, we use birth-death models to quantify the strength of selection for cheating required to generate the patterns of cheat diversity and abundance that we see. This reveals that for some host species, viral cheats could be driving cooperator viruses extinct over the course of an infection, in a way that is analogous to the emergence of cancer in multicellular organisms. Thirdly, we test for a genetic basis of susceptibility to cheating, finding that cheat abundance is phylogenetically conserved. This indicates that standing genetic variation exists for susceptibility to viral cheating within circulating H5N1 lineages, suggesting conflicting selection pressures at the within- and between-host level.

Overall, our findings suggest that: cheats are highly abundant in natural influenza infections of diverse animal species; new large viral sequencing datasets call for new mathematical and statistical approaches; the resulting evolutionary insights reveal the previously unseen social complexity of viral infections.



SAI: A tool for Statistics for Adaptive Introgression

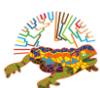
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POSTER SESSION 1 - P01.039, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Adaptive introgression is an important evolutionary process, for which an approach has been developed based on summary statistics, such as the number of uniquely shared sites and the quantile of derived allele statistic. However, no accessible implementation has been available, limiting reproducibility and methodological clarity. Here, we present SAI, a package for computing these in a flexible and robust manner, which allows an easy application to different datasets. We demonstrate using the 1000 Genomes Project data, that SAI can replicate previously reported candidate regions based on these statistics, and identify additional ones, including a region

detected using a deep learning method. Furthermore, we show that SAI can be applied to non-human diversity datasets to detect adaptive introgression. We investigated bonobo introgression into central chimpanzees, providing candidate regions that might have been beneficial in chimpanzees after gene flow. Interestingly, one candidate region was also found at high frequency in modern Papuans and assigned to Denisovan adaptive introgression. Such co-occurrence suggests that similar genes might have been beneficial after introgression in different species.



Semi-supervised selection target detection with positive-unlabeled learning

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POSTER SESSION 1 – P01.030, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Identifying genomic regions shaped by natural selection is a central goal in evolutionary genomics. Existing machine learning methods for this task are typically trained using simulated genomic data labeled according to specific evolutionary scenarios. While effective in controlled settings, these models are limited by their reliance on explicit class labels. They can only detect the specific processes they were trained to recognize, making it difficult to interpret predictions for regions influenced by other evolutionary forces. This limitation is especially problematic when analyzing empirical genomes shaped by a mixture of adaptive, demographic, and ecological factors. One-vs.-rest strategies offer a potential alternative, but suffer from the inherent complexity of modeling all other evolutionary and demographic processes as a catch-all "rest" class. Here, we explore positive-unlabeled learning as a more flexible framework for detection of adaptive events. Positive-unlabeled learning is a semi-supervised approach that permits identification of samples of a target class using only positive labels and an unlabeled background, without requiring explicit modeling of negative samples. To assess the utility of this approach, we focus on a binary

classification setting for detecting selective sweeps (positive samples) arising from positive natural selection against a mixed background composed of both unlabeled sweeps and neutrally-evolving regions. To accomplish this goal, we introduce PULSe, a method that employs only a set of labeled sweep observations for training while treating all remaining data as unlabeled. By avoiding assumptions about the composition of the background, PULSe enables robust sweep discovery in realistic genomic landscapes. We systematically evaluate its performance across a range of demographic, adaptive, ecological, and confounding contexts, including domain shift arising from misspecified demographic models, and find that PULSe delivers high performance and generalizability. To demonstrate a practical application of PULSe, we analyzed European human genomes, treating the empirical genome as the unlabeled set, and recapitulating several previously-identified sweep candidates. Our results show that PULSe provides a powerful, and versatile alternative for detecting adaptive regions, with the potential to generalize across a range of genomic landscapes, including non-model organisms.



Species trait data imputation via deep learning

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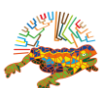
POSTER SESSION 1 – P01.026, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Species traits are the morphological, demographic, physiological, phenological, and genetic features of a given species, and are used in studies such as biodiversity forecasting, parameterising process-based models, and investigating species evolutionary histories, as well as in conservation projects. These data often display high degrees of missingness, and as such effective data handling techniques are essential to maximize data availability. One such approach is data imputation, in which a most probable value is determined for missing data.

Current approaches for imputing missing species trait data often focus on applying statistical and simple machine learning approaches such as random forest. Deep

learning approaches offer a potentially powerful alternative to these traditional methods due to their capacity to ‘understand’ complex relationships within large datasets.

Here, we present our work on the development of deep neural network models for imputing missing species trait data, including the training and application of deep neural networks and foundational models. We also fine-tune a pre-trained foundational prior fitted network for the same task. These models have the potential to provide significant performance improvements over conventional trait data imputation approaches under a variety of missingness data scenarios, providing researchers with more robust trait datasets.



The avian colourscape and its uncertain future

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POSTER SESSION 1 – P01.029, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The profusion of animal colour – from birds to butterflies – is one of the most striking aspects of biodiversity, and something we as humans intrinsically value. But is this diversity under threat as our environment changes and species face extinction? One barrier to understanding and mitigating this impact is the difficulty in quantifying diversity in holistic colour pattern phenotype, a notoriously complex trait. Here, I explore our

work leveraging recent advances in machine-learning-based computer vision techniques to quantify current and future diversity in colouration in birds, a famously colourful group of animals. We find that avian colour pattern diversity may be at risk of homogenisation from anthropogenic extinctions, with the loss of threatened species leading to disproportionate reductions in colour diversity.



The friendship paradox and social structuring in animal societies

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POSTER SESSION 1 – **P01.048**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: A population's social structure, or 'social network', shapes various biological processes, including the spread of disease, information, and behaviour. How social structure arises governs how these contagions spread. One network phenomenon of interest is the 'friendship paradox' – where on average an individual's connections have more connections than they do. It is a way of measuring relationship disparity within and across a social network, and thus how social structuring across species leads to asymmetric social experiences. By quantifying this relationship disparity across networks, we suggest a new metric to use to understand the trade-off behind social interactions. Understanding what network and biological features are associated with this disparity is important for uncovering the evolutionary pressures shaping social behaviour, yet there is currently little understanding of how it varies across real-world social networks, and what biological or network properties can exaggerate it or diminish it.

Materials and methods: We analysed 399 wild animal social networks to consider how the strength of the friendship paradox varies

with intrinsic network characteristics and biological attributes. We then applied a null modelling approach to examine whether the friendship paradox was accentuated more than expected given network structural features.

Results: Several key network structures and biological features predicted the strength of the friendship paradox, with larger and denser networks generally exhibiting a weaker friendship paradox, and mammals and birds showing a stronger relationship disparity. We found that mammalian and reptilian networks had less disparity than expected from their network structure.

Conclusions: These findings highlight how application of network theory can reveal insights into underlying principles of social structure. This study demonstrates how applying computational and network-theoretic tools in a comparative study can uncover general patterns in social structuring across species. It highlights the potential of network-based modelling to bridge behavioural ecology and evolutionary theory.



The Inference Challenge: Regularization Methods Across Computational Paradigms in Evolutionary Ecology

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POSTER SESSION 1 – P01.037, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

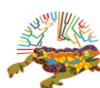
In the era of big data and machine learning, evolutionary ecologists face a fundamental tension between predictive power and causal inference. This methodological work explores this inferential challenge through regularization strategies across both classical statistical and machine learning approaches applied to multivariate trait data in comparative phylogenetics and population genetics.

We compare regularization philosophies for detecting sparse signals in correlation structures: traditional Bayesian priors, sparsity-inducing regularization (horseshoe and adaptive lasso), and dimension-reduction techniques. Using Stan, we implement these with optimized likelihood calculations for tree-based data structures. We also explore neural network architectures that incorporate structured biological dependencies through appropriate distance metrics.

These approaches highlight contrasting validation philosophies: classical statistical methods offer rigorous parameter uncertainty quantification using posterior

distributions or confidence intervals as the foundation for inference, while machine learning primarily validates through predictive performance, with limited tools for assessing uncertainty in internal parameters or post-hoc explanations. The dual challenge across computational paradigms is managing model complexity: insufficiently regularized models risk hallucinating spurious patterns, while excessive regularization can eliminate genuinely sparse but important signals—both undermining valid inference in complex evolutionary systems.

Our preliminary results based on simulation studies in the classical domain suggest that targeted regularization approaches like horseshoe priors show promise in recovering sparse (partial) correlations in multivariate tree-structured trait data by adaptively determining where complexity is needed. Using simulated and real data sets, our results demonstrate how different regularization strategies represent inherent trade-offs between interpretability, computational efficiency, and inferential validity in complex evolutionary models.



The Power of Three: Enhancing Arthropod–Plant Interaction Monitoring with Citizen Science, Expert Taxonomic Criteria, and Computer Vision Models

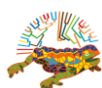
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POSTER SESSION 1 – P01.018, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The coevolution between flowering plants and their visitors has charmed evolutionary biologists for centuries. Yet, data on reproductive biology remain sparse for most plant families—especially those considered to have generalist pollination strategies, such as the Asteraceae, one of the most speciose angiosperm families. Here, we present a pipeline for processing video-based floral visitor data, built around a custom YOLO (You Only Look Once) object classification model. This approach combines citizen science, expert taxonomic input from botanists and entomologists, and computer vision to automate and scale up the monitoring of plant-arthropod interactions. Pipeline Overview: Model Preparation—(i) We compiled a species list of arthropods from the Pyrenees based on two curated iNaturalist projects focused on Asteraceae interactions, yielding over 7,000 observations (covering ~600 arthropod and 150 Asteraceae species). (ii) We then assembled a dataset of 399,460 images across 330 arthropod species sourced from GBIF (Global Biodiversity Information Facility), excluding taxa with insufficient image data. (iii) This dataset was used to train and validate a custom YOLO model, achieving an 81% precision for the top

predicted class and 90% for the top five predictions. (iv) To improve classification reliability, we developed a performance index combining prediction confidence and consistency across the top predictions. Video Analysis—(v) We processed over 800 hours of video from ten Asteraceae species in the Pyrenees, extracting one frame per second. (vi) The YOLO model was applied to each frame, generating summary tables of interaction times, predicted taxa, and performance scores. Key frames were sorted into folders for expert review. Model Validation—(vii) An entomologist evaluated a subset of predictions and conducted direct video assessments to identify potential biases—such as false positives, overlooked species that were not part of the training dataset, or taxa undetected in the automated analysis. This study highlights the promise of computer vision in overcoming long-standing data limitations in the study of plant-arthropod interactions. While challenges remain—particularly in generating high-quality training data and ensuring accurate species recognition—our results show how citizen science and expert knowledge can be effectively combined to advance ecological research at scale.



The Role of Robustness in Shaping Metabolic Network Evolution Across Species

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POSTER SESSION 1 – P01.042, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

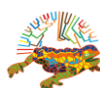
Biological systems face the dual challenge of maintaining stable function in the face of perturbations while also adapting to new environments and evolutionary pressures. This balance between robustness and evolvability is particularly evident in metabolic networks, which must resist disruption yet remain capable of innovation. We propose a framework to investigate how metabolic networks navigate this trade-off by combining systems biology approaches with evolutionary analysis.

Specifically, and in contrast to prior work, we assess robustness as well as function similarity of metabolic networks using structural sensitivity analysis applied to genome-scale metabolic models. Structural sensitivities identify reactions whose perturbation—such as changes in enzyme activity or gene expression—has significant effects on overall network function. By quantifying the sensitivity of individual reactions, and the correlations of sensitivities between networks of different species, we can determine which parts of the networks are most functionally similar and most critical to maintaining stability.

In a first step to explore the evolutionary consequences of this sensitivity, we propose comparing the network's sensitivity to internal (mutational) versus external (environmental) perturbations to assess whether robustness has evolved more strongly in response to one type of challenge.

This includes examining whether evolutionary rates of enzymes differ depending on the type of perturbation to which they are most sensitive, helping to identify dominant sources of selective pressure. In terms of sensitivities, we find slightly higher values for the perturbation of exchange reactions. When we use sensitivity profiles to reconstruct phylogenetic trees—based either on internal or external perturbations—we obtain different topologies, indicating that the type of perturbation considered influences the inferred evolutionary relationships. We will relate this to mutation rates (dN/dS) to explore how selective pressures have shaped these trends.

Our framework aims to shed light on the fundamental principles governing how metabolic systems remain both stable and adaptable under evolutionary pressure by assessing network function via structural sensitivities, without the need for detailed (evolutionary) models. We consider our first results regarding differential sensitivity to internal and external perturbations – and their impact on phylogenetic inference – as promising in this direction. In the future, to investigate other aspects of long-term evolutionary dynamics, for example, we will examine the fate of duplicated metabolic genes, considering that gene duplication is a major source of innovation.



When barriers break: the probabilistic assembly of pathogen's host-range and the percolation of epidemics

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POSTER SESSION 1 – P01.047, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Understanding how changes in pathogen host range influence disease spread is essential for anticipating large-scale outbreaks. Percolation theory, traditionally used in physics, has recently been applied to ecological networks to explain sudden transitions from localized outbreaks to widespread epidemics. However, little attention has been given to how evolutionary changes in pathogen composition interact with the host contact network to trigger percolation. Here, we integrate evolutionary and ecological theory to investigate how percolation emerges from the evolutionary dynamics of a pathogen's host range.

Materials and Methods: We developed a probabilistic model of pathogen evolution and transmission across a weighted, directed host-contact network. Each node represents a host species population of 100 individuals. Edge weights define the probability of contact between nodes. Pathogen strains are characterized by vectors of host-specific capacities determining replication and infection success. At each time step, infected individuals release propagules, which disperse through the network according to normalized edge weights, forming the ecological opportunity for spread. For each potential host, the number of encounters with propagules is drawn from a binomial distribution, based on propagule load and contact probability. A second binomial process determines which encounters result in infections, based on the pathogen's capacity in that host. Within hosts, strains compete through deterministic exclusion: only the most competitive strain (with highest capacity) persists. Evolution occurs through mutations during infection: new strains arise via random mutations in capacity; capacities to other host species are influenced by trait correlations, capturing trade-offs and pleiotropic constraints. This framework captures both ecological transmission and evolutionary diversification, accounting for phylogenetic conservatism, host heterogeneity, and network structure.

We ran a total of 96 simulations across multiple contact networks, tracking the emergence of new variants, their transmission, and the resulting dynamics of spread and percolation.

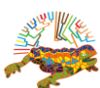
Results: Across simulations, percolation consistently followed host-range expansion into new network modules. This suggests modular boundaries act as ecological barriers, and that percolation arises through a synergistic build-up of propagule pressure across modules, rather than within a single compartment.

Percolation was typically preceded by infection of a high-capacity host in another module. These key hosts act as evolutionary amplifiers, enhancing persistence and increasing the chances of cross-module transmission.

Percolation consistently occurred only after the total number of infected individuals reached a critical abundance threshold, which remained relatively stable across network structures. This suggests a minimum infection load is required to trigger percolation, reflecting a fundamental constraint in transmission dynamics.

Finally, percolating variants most often emerged in their ancestral (reservoir) host, likely due to a combination of higher evolutionary capacity and sustained transmission amplification in that host.

Conclusions: Our results show that epidemic transitions are shaped by the interaction between evolving host range, modular host community structure, host-specific capacities, and temporal variation in transmission. Unlike traditional models based on stochastic transitions between network states, our framework captures the continuous spread of propagules governed by biologically grounded parameters. This propagation-based approach offers a mechanistic and evolutionary perspective on how pathogens overcome barriers to colonize new hosts. By integrating evolution into network epidemiology, this study provides a generalizable framework for predicting pathogen emergence and identifying the ecological conditions driving epidemic spillovers.



Eco-evolutionary dynamics of finite populations from first principles and the noise-induced reversal of natural selection

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MEETING ROOM 129+130, August 19, 2025, 11:00 AM - 1:00 PM

While we understand evolution in infinitely large populations in a remarkably general manner through frameworks such as the Price equation, finite populations are considerably more complex. Standard models in population genetics, such as the Wright-Fisher or Moran model, assume the total population size is either strictly constant or varying according to externally imposed 'top-down' rules, thus neglecting ecological dynamics and density-dependent demographic effects. In this talk, I will show how modern tools from stochastic calculus and Markov chains can be used to analytically derive equations for the eco-evolutionary dynamics of finite populations from demographic first principles. Our equations generalize and extend well-known descriptions of evolution, such as the replicator equation, Price equation, and Fisher's fundamental theorem, to finite populations of non-constant size. Our framework also reveals how stochasticity in finite populations can predictably bias evolutionary trajectories to favour certain traits, a phenomenon we call "noise-induced biasing". We show that noise-induced biasing acts through two distinct mechanisms, the "direct" and "indirect" mechanisms. While the direct mechanism can be identified with classic bet-hedging theory, the indirect mechanism is a more subtle consequence of frequency- and density-dependent demographic stochasticity. Our equations reveal that noise-induced biasing may lead to evolution proceeding in a direction opposite to that predicted by selection-mutation-drift balance alone in finite populations, helping explain several recent studies showing counter-intuitive effects of stochasticity on evolutionary dynamics.

I will also sketch how one can carry out an analogous derivation for populations bearing quantitative traits. Modelling the complete dynamics of quantitative trait distributions in finite populations is mathematically challenging since infinitely many distinct trait variants may

arise in such populations. I will show how tools from stochastic field theory and infinite-dimensional stochastic analysis can be used to derive the complete dynamics of the distribution of a quantitative trait in a finite population of non-constant size from first principles. The resulting equations extend classic results from quantitative genetics, such as the Crow-Kimura equation and Lande's gradient equation, to the stochastic, dynamic setting relevant to finite populations. On the mathematics side, the work on quantitative traits also introduces new mathematical techniques for modelling stochastic quantitative trait evolution using stochastic partial differential equations (SPDEs). These new mathematical techniques complement the existing standard approach in the literature, thus expanding the theoretical toolbox to model quantitative traits in finite populations. As an application, we will see how the SPDE approach allows us to use Fourier techniques to study adaptive diversification in finite populations.

Several theorists have called for a reformulation of eco-evolutionary dynamics from the demographic first principles of birth and death rates because such a formulation is more fundamental and 'all roads to fitness lead through demography'. Our work provides a starting point for such a reformulation, using modern mathematical tools to generalise some fundamental equations of eco-evolutionary theory. The idea of noise-induced biasing also conceptually unifies a considerable number of existing studies under a single theoretical framework.

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How does species diversity affect ecological stability?

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MEETING ROOM 129+130, August 19, 2025, 11:00 AM - 1:00 PM

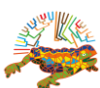
Background: The diversity-stability relationship remains a foundational yet contentious topic in evolutionary ecology. Resolving how species diversity impacts ecological stability is critical for understanding ecosystem resilience in a rapidly changing world.

Materials and Methods: We developed a generalized mathematical framework that integrates canonical ecological models—including generalized Lotka-Volterra, consumer-resource, and sublinear models—by explicitly incorporating nonlinear growth functions defined by density-dependent per capita growth rates. We systematically examined how different functional forms of the growth function influence stability outcomes across diverse parameter spaces.

Results: Our theoretical analysis revealed that the diversity-stability relationship

depends fundamentally on the shape of the growth function. Specifically, we demonstrated that increasing species diversity always destabilizes ecological communities, unless (1) intraspecific interactions exhibit specific decelerating nonlinear density regulation, and (2) interspecific interactions follow linear or accelerating regulations. Empirical analyses on macroecological and microbial data further support the dominance of destabilizing patterns.

Conclusions: This study demonstrates how advanced mathematical modeling and computational methods can reconcile longstanding debates in ecological theory. Our results highlight critical conditions under which species diversity can enhance stability, offering theoretical insights crucial for understanding ecosystem resilience.



Inference of ecological and life-history traits evolution from genomic and epigenomic polymorphism data.

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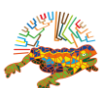
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MEETING ROOM 129+130, August 18, 2025, 10:30 AM - 12:30 PM

While most population genetics inference methods using full-genome data can be, in principle, applied to all species, the underlying assumptions of classic models of population genetics are often sexual reproduction in each generation and non-overlapping generations. However, in many plants, invertebrates, fungi and other taxa, those assumptions are often violated due to different ecological and life history traits, such as self-fertilization, long term dormant structures (seed or egg-banking) or large variance in offspring production. Moreover, these life-history traits do evolve themselves in response to changing climate and ecology of the species. In return life-history traits do determine species genetic diversity and evolutionary potential to adapt to biotic and abiotic changes. Yet, the evolution of such life-history traits has been chiefly studied using phylogenies over macroevolutionary time scales, preventing to study recent trait transitions and the underpinning neutral and selective mechanisms. Furthermore, the resolution of past population genetics inference decreases when there is a lack of SNPs in the data, decreasing the resolution of inference in recent times, especially recent effects of climate change. Technological advances allow now to use genomes and epigenomes (methylation data) for model and non-model species to

decipher the evolutionary processes driving ecological adaptations.

Building on adapted coalescent models, we have developed novel inference methods which aims at estimating transitions in life-history traits using full genome polymorphism data. We developed Sequential Markovian Coalescent (SMC), Approximate Bayesian Computation (ABC) as well as Deep Learning (DL based on large language models) methods to 1) infer transitions in seed banking / dormancy or selfing rates in time, and 2) integrate epigenetic (methylation) markers to improve the inference of recent events. We provide here an overview of the statistical power and robustness of these various methods in estimating and disentangling between demographic events and trait transitions. We then explicit the footprints of these trait transitions in the genome wide polymorphism data and how to detect them using statistics such as transition matrices summarizing times to the most recent common ancestor. We explicit the respective contributions of SMC, ABC and DL methods to our understanding of the heterogeneity in genomic footprints of trait transitions. We finally highlight the novel insights gained with DL methods to decipher the evolutionary processes driving the evolution of ecological/life-history traits.



Neural ODEs for understanding population dynamics

Patel M¹

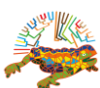
¹University Of Hull

MEETING ROOM 129+130, August 19, 2025, 11:00 AM - 1:00 PM

Ordinary Differential Equations (ODE) are a widely used tool in understanding dynamics of systems. In Evolutionary-ecology they are used in population dynamic modelling and most relevantly for eco-evolutionary processes in adaptive dynamics approaches. Neural Ordinary Differential Equations (NODEs or Neural ODEs) apply the concept of neural operators to differential equations to learn the differential equations of a system from data. This is a Data driven approach to understanding the dynamics of a system. In this talk I hope to give anyone familiar with the basic concept of an ODE an intuition for what a neural ODE is doing and how to use them to unravel the ecological dynamics of a system. I will present some applications of neural ODEs to long term time series data of species from the Global

Population Dynamics Dataset. And give a proof of concept of how they can be used with existing datasets to better understand complex ecological dynamics. This is still a relatively young field and I believe there are many challenges to implementing neural ODEs robustly. My hope is this talk will widen interest in using neural ODE and spark further discussion in the literature on how to use neural ODEs to best effect.

The talk will give a simple introduction to how neural networks and ODEs can be integrated. How training is done including common packages in Python and Julia to do this training. And some explorations of how to recover biological insights from the trained neural networks so they are not being used as simple black boxes.



Neural posterior estimation for high-dimensional simulation data from complex ecological and population genetic models

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MEETING ROOM 129+130, August 18, 2025, 10:30 AM - 12:30 PM

Quantifying the evolutionary forces that shape natural populations is a key challenge in evolutionary ecology, where accurate inference improves our understanding of eco-evolutionary dynamics and local adaptations. Simulation-based inference has shown great potential and become increasingly useful in this field because they accommodate biologically realistic models when likelihoods are intractable. Approximate Bayesian Computation (ABC) is a widely used simulation-based inference method, but comes with substantial computational expense and struggles to fit models to high-dimensional summaries of the data now routinely collected in ecological studies. In contrast, supervised Machine Learning (ML) techniques are capable to handle such high-dimensional data. However, ML methods generally do not yield Bayesian uncertainty estimates of the quantities they predict. Here, we present neural posterior estimation (NPE), a deep-learning framework that combines the best facets of ABC with the efficiency of supervised machine learning. By training

neural density estimators on simulated data, NPE directly approximates the full posterior distribution of model parameters.

Benchmarking across a variety of population genetic tasks shows that the neural posterior estimators yield posterior distributions with high accuracy and better efficiency than ABC or parametric bootstrapping. We use the 2 epoch demographic model of *Arabidopsis thaliana* to estimate the timing and magnitude of a bottleneck event. We also demonstrate that incorporating linkage-based statistics alongside classical site frequency spectra improves the prediction accuracy in certain settings. Additionally, we apply neural posterior estimation for demographic inference for simple and more complex models to highlight its application. For organisms with the sub-population structure, the method can also be utilized to investigate migration events. Finally, we provide a user friendly workflow that enables others to perform neural posterior estimation to their own genetic data.



Phylogenetic probabilistic PCA

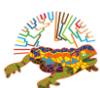
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MEETING ROOM 129+130, August 19, 2025, 11:00 AM - 1:00 PM

It is common practice in evolutionary biology to study the evolution of phenotypes from multiple traits. Then, principal component analysis (PCA) is probably the most commonly used technique to summarise the information in a few variables, making easier its handling and interpretation. For comparative data, a phylogenetic version of PCA is needed to take into account the relationship between species and to avoid spurious conclusions. However, to apply phylogenetic PCA, the evolutionary model underlying the data set must be defined, which is usually unknown, and only a Brownian motion process can be used when the number of variables exceeds the number of lineages. This becomes a limitation since the reliability of the conclusions depends on the PCA being performed under the correct evolutionary model. To overcome this limitation, we implemented a probabilistic PCA in a phylogenetic framework. This technique, phylogenetic probabilistic PCA (P3CA), is developed from a continuous latent variable model where it is possible to evaluate a likelihood function for a multivariate normal distribution. This allows to evaluate the fit of multiple evolutionary

models to the data and then, without the need for assumptions. Also, we formulate an Expectation-Maximisation (EM) algorithm, which potentially makes it faster to reach a solution, but also allows to deal with missing cases. Using the implementation for Pagel's lambda model and simulations, we evaluate the accuracy of the P3CA and find a high accuracy when a complete dataset is used in both, when the number of lineages exceeds and is exceeded by the number of variables. If missing cases are included (up to 10%), the evolutionary parameter describing the model is slightly underestimated. This underestimation still allows to recover the information in the new reduced space, measured by the similarity between the loadings. To illustrate, we applied the P3CA to geometric morphometric datasets describing the skull shape of crocodylians and bats. We expect that having the formulation for phylogenetic PCA based on a probabilistic, to address some of the limitations for using this primary dimension reduction technique in the study of phenotypic evolution, helping to deal with and understand the large volumes of high-density data currently being generated



The evolution of reversible plasticity in stable environments

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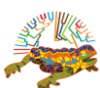
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MEETING ROOM 129+130, August 19, 2025, 11:00 AM - 1:00 PM

Reversible plasticity, i.e. the ability to deconstruct phenotypic specializations based on environmental conditions is widespread in nature. Despite its ubiquity, few mathematical models have explored the evolutionary selection pressures that favor trait reversibility. Here, we present a model of the evolution of reversible plasticity in which organisms incrementally tailor phenotypes to their environment based on cues. Contrasting previous work the environmental state varies between but not within generations. We highlight two results. First, although plasticity in construction is

typically highest early in ontogeny, we observe the highest levels of plasticity in deconstruction in mid-ontogeny. Second, we find that reversibility often evolves in ontogenetically stable environments. Thus, contrasting common beliefs, our model shows that reversibility does not require environmental change during development—as long as organisms are initially uncertain about environmental conditions. Our model provides new insights into the capacity for reversibility in species that have evolved in ontogenetically stable environments.



The value of ‘simple’ models in the era of complexity: Case studies from sexual selection

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MEETING ROOM 129+130, August 19, 2025, 11:00 AM - 1:00 PM

Access to technological advances and ever more complex models provides opportunities we could previously only dream of. However, this does not imply that simpler models and classical modelling methods have become obsolete. I argue for the opposite case: that instead of moving away from simple methods, the increasing prevalence of very complex models should be accompanied by attempts to understand root causes of phenomena using classical and often simple approaches. In the best-case scenario, distilling models into the simplest possible forms can yield insight regarding biology and causality that is inaccessible using very complex models. I

briefly discuss examples from past decades where a very simple model has brought clarity to a range of complex models. I then turn to my recent work on sexual selection, where I have used simple models to bring clarity to debated theoretical issues, or to controversial results arising from large-scale data analysis. While simplicity of a model does not guarantee truth, if two theories are otherwise on an equal footing, the simpler ones, explaining the most using the least are typically preferable. I argue that sometimes we can be blinded from both the right questions and from the right answers if the focus is solely on complex methods



The variance world: the era of location-scale models in evolutionary meta-science

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MEETING ROOM 129+130, August 18, 2025, 10:30 AM - 12:30 PM

Understanding how both the mean and variance of traits differ among species and lineages is fundamental to unveiling macroevolutionary patterns. Yet, traditional phylogenetic comparative methods primarily focus on modelling mean trait values, often overlooking variability and heteroscedasticity that can provide critical insights into evolutionary dynamics. We introduce phylogenetic location-scale models (PLSMs) and location-scale meta-analyses, a novel framework that jointly analyzes the evolution of trait means and variances. This dual approach captures heteroscedasticity and evolutionary changes in trait variability, allowing for the detection of clades with differing variances and revealing patterns of adaptation, diversification, and evolutionary constraints. Extending PLSMs to a multivariate context enables simultaneous analysis of multiple traits and their covariances, facilitating the testing of hypotheses about evolutionary

trade-offs, pleiotropy, and phenotypic integration. By modelling covariances between phylogenetic effects in both the location and scale (variability) parts, we can discern whether changes in one trait's mean or variance are associated with changes in another's, thereby offering deeper insights into the mechanisms driving trait co-evolution, and co-divergence or "contra-divergence". We also describe how an extended version of PLSMs incorporating within-species variability can enhance our understanding of trait convergence and divergence arising from ecological and environmental factors. Our framework provides an innovative and flexible tool for exploring macro-evolutionary patterns by jointly modelling trait means and variances. Importantly, PLSMs can be used to reassess almost all previously published comparative data, providing new evolutionary insights and enriching our understanding of the diversity of life.



Trend-filtered support vector machine to detect natural selection from genomic autocovariation

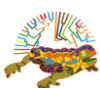
Amin M¹, DeGiorgio M

¹Florida Atlantic University

MEETING ROOM 129+130, August 18, 2025, 10:30 AM - 12:30 PM

A major challenge in evolutionary genomics is accurately identifying and characterizing natural selection from genomic data. The wide availability of dense whole-genome datasets has enabled researchers to analyze and localize genetic variation within populations. Powerful supervised machine learning methods allow researchers to extract spatial information about genetic variation across the genome and detect traces of natural selection in light of the many genetic, demographic, and ecological influences that might mask adaptive signatures. However, methods such as convolutional neural networks do not preserve spatial information and often suffer from high computational complexity due to their large number of parameters. In contrast, trend filtering directly models the autocovariation of neighboring features, ensuring that spatial relationships remain intact. When integrated into a standard linear model, such as a support vector machine, trend filtering offers a natural framework to create powerful predictive models while retaining the spatial integrity of the input. Here, we introduce SKINET, which employs a

novel trend-filter kernel within a support vector machine framework and applies it to the task of detecting and characterizing regions affected by positive natural selection. This new kernel that we mathematically derived can be readily plugged into a standard SVM classifier or regressor framework, and represents a powerful extension of a linear model that provides prediction performance of natural selection comparable to contemporary deep learning-based methods. Specifically, SKINET not only distinguishes regions under positive natural selection from neutrally evolving regions but also functions in a regression framework to estimate associated adaptive parameters. Moreover, applying SKINET to empirical human genome variation identifies adaptive candidate genes consistent with previous findings while also uncovering novel adaptation targets (e.g., FAM177A1) linked to cancer. Though illustrated on human genomic data, SKINET flexibly handles summary statistic and unphased multilocus genotypes as input, making it suitable for application to non-model organisms.





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**CONGRESS OF THE EUROPEAN SOCIETY FOR
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S04 - Ageing outside of the box: insights from unusual and non-model species



A life history model of continuous growth and negligible senescence under resource allocation trade-offs

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POSTER SESSION 2 - **P02.012**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The evolution of aging remains as one of the unanswered questions in evolutionary biology. Long-lived organisms tend to be characterized by prolonged growth periods or even indeterminate growth, in which growth continues throughout their lifespan. We present a mathematical model with resource allocation schedules as evolving traits that explores the connection between indeterminate growth and negligible and negative senescence. By synthesizing and developing previous theoretical work, we show that the indeterminate growth does not lead to negative senescence as was previously thought. Instead, under different ecological and physiological conditions, optimal somatic maintenance allocation can

lead to diverse age-trajectories of mortality that include slow senescence and sustained allocation to growth. Our work shows the connection between growth strategies, mortality and fertility rates, and details optimal allocation strategies that can lead to even more complex nonmonotonic mortality patterns. While we do not focus on human specific life histories (such as intergenerational transfers), this work also provides clues for the type of ecological and physiological conditions that promote longevity. These findings clarify the role of growth schedules and other ecological and physiological conditions that favour the evolution of ultimately longer lifespans.



A phylogenetic analysis of sporocarp longevity and alternate life history strategies in Polypore mushrooms

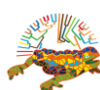
Venkatraman G¹, James T¹

¹University of Michigan

POSTER SESSION 2 - **P02.011**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Senescence in mushroom-forming fungi (Agaricomycetes) has received limited attention despite many macrofungi having the potential for infinite hyphal growth as evidenced by the 1500 year old Humongous Fungus (*Armillaria gallica*) and the fairy ring mushroom, *Marasmius oreades*. Fruiting bodies produced by these long-lived fungal species and most Agaricomycetes in general, however, are temporary structures with marked senescence and mortality. The mushroom is often the only part of the fungus that shows degeneration, surviving and remaining functional from a few days to a couple of weeks on average. While certain species invest in numerous small fruiting bodies in clusters, others produce single to a few fruiting bodies ranging in body size from a few millimeters to a meter. Irrespective of which of the aforementioned strategies they follow, most macrofungi invest in ephemeral fruiting bodies, producing new ones each growth season. Interestingly though, certain species within Polyporaceae and

Hymenochaetaceae (commonly referred to as polypores), have diverged from this trend and instead invest in a single or a few limited perennial fruiting bodies and maintain these fruiting bodies over several growing seasons. Every growing season, these fungi produce a fresh hymenium or fertile, spore-producing layer on top of the pre-existing hymenophore. In other words, they invest and maintain a single or a small, limited number of fruiting bodies throughout their life. This marked difference in life-history strategy seems to have evolved only among polypores, many of which also produce hard fruiting bodies with structural tissue differentiation. Given the occurrence of long-lived sporocarps in some polypore species, here, I present results from ancestral state reconstructions of polypores to identify gains and losses of this alternate life history strategy, determine if it has a phylogenetic signal and its potential link to the anatomy of the sporocarp.



Age-related changes and selective disappearance shape variation in bold-shy continuum in guppies

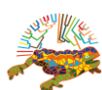
Herdegen Radwan M¹, Raubic J¹

¹Adam Mickiewicz University

POSTER SESSION 2 - P02.009, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The persistence of animal personalities within populations contradicts the optimality principle, assuming a single optimal trait value for all individuals. With the advance of research on the maintenance of animal personalities, two types of data have emerged as particularly needed: (1) longitudinal data, allowing to distinguish population-level and cohort-level variance in personalities, and (2) estimates of additive genetic variance in personality and their genetic correlations with life history traits. While longitudinal studies are beginning to emerge, most research to date has relied on personality measures at a single life-stage, limiting our understanding of the interplay between ages and genotypes. To address this gap, we employed a three-generations pedigree design in a captive guppy (*Poecilia reticulata*) population, measuring boldness at different stages of ontogeny and collecting

long-term survival data. This design enabled us to investigate the genetic contribution to variance in both boldness and survival and examine how this contribution changes across the lifespan in the personality trait. Our results show that boldness decreased with age in genotype-dependent manner. Additionally, boldness was negatively associated with survival. However, while additive genetic effects contributed to variation in boldness, they did not contribute to survival, indicating no genetic basis for the boldness-survival correlation. Lastly, males exhibited higher, more consistent boldness than females. At young ages, the variance in male boldness was primarily driven by additive genetic effects, while maternal effects were more prominent in females. Overall, our findings highlight the complex, dynamic interplay of age, genotype, and sex in shaping individual behaviour.



An Evolutionary Origin of Ageing: Jensen's Inequality and Asymmetric Division in Unicellular Life

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POSTER SESSION 2 - **P02.016**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Ageing appears intrinsic to unicellular life: subtle within a single cell, yet with complex and variable form across lineages and environments. In model microbial species, such as *E. coli* and *S. pombe*, there is a subtle 'asymmetry' in division where daughter cells consistently differ in growth and survival rates due to unequal inheritance of cellular components. With binary fission, damage accumulates at cell poles and is unevenly inherited by 'old-pole' daughters relative to 'new-pole' daughters. Repeated asymmetric division produces a fractal-like age distribution, with notable plateauing declines along the old-pole lineage. Despite empirical progress, it remains unclear why multiple forms of asymmetry are inherent to cell division, each with distinct structure and variation.

Here, we provide a theoretical synthesis of adaptive evolution of microbial ageing and model transgenerational selection pressures involving multiple inherited components. By extending prior theoretical frameworks (Ackermann et al., 2007; Chao, 2010), we first propose Jensen's inequality as the fundamental criterion for adaptive asymmetric reproduction, applicable to both damage and beneficial components.

1. Jensen's inequality: For a set of values, the average of a convex function applied to each value is greater than the same function applied to the average of the values.

2. Asymmetry definition: Daughter cells inherit opposing deviations in cellular components, creating variance in lineage-specific fitness centred relative to symmetric division.

3. Adaptive Criterion: Asymmetry is selected only when the convex fitness advantage gained by one daughter outweighs the convex fitness loss suffered by its sister, balanced against partitioning costs.

4. Conclusion: Adaptive evolution of microbial ageing inherently depends on convex inheritance-fitness relationships.

We examine how curvature in individual performance functions transforms into lineage-specific fitness, demonstrating how convexity emerges naturally under ecologically plausible selective conditions. This allows asymmetry to evolve even when individual-level

performance functions are linear or concave. For example, cellular survival probabilities with a negative linear relationship to damage transform into a convex lineage-level fitness relationship, due to multiplicative fitness effects across generations. Advances in microfluidics and molecular tagging now enable lineage phenotyping of cell performance and inheritance, allowing for inference of curvature in performance-inheritance functions.

For more complex selective conditions, we analyse the Hessian matrix of lineage-specific fitness and inheritance functions, linking local curvature to fitness outcomes via eigenfunctions of lineage projection kernels. Using an analytical reduction to isolate transgenerational effects, we quantify the local reproductive value of asymmetric division and demonstrate adaptivity across global age distribution bounds.

Interactions: A single heritable component can have multiple interactive effects on fitness landscape curvature, such as damage correlated with division and survival rate. Multiple heritable components can also interact, selecting for 'associativity' in asymmetry, where components become coupled or decoupled in their inheritance.

1. Selection events: Bottlenecks selectively remove low-performance cells, amplifying small trait differences and inducing convexity in fitness through time.

2. Variability in accumulation rates: Non-constant accumulation per generation produces multiplicative, transgenerational lineage effects that favour asymmetric division.

3. Homeostasis: Population growth depends on a concave averaging of division times, with selection to reduce division-time noise. This favours asymmetry in homeostatic control, with tighter regulation in higher-performance cells.

Together, convexity emerges naturally over multiple generations, transforming individual-level performance into lineage-specific fitness, with interactions driving directional associations among asymmetric components. Structured damage asymmetry therefore provides a foundation for phenotypic differentiation across diverse forms of inheritance.



Asymmetric life history trade-offs shape sex-biased longevity patterns

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POSTER SESSION 2 - **P02.015**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Patterns of sex biases in mortality and lifespan vary widely across the animal kingdom, yet there is limited consensus on their underlying mechanisms. A major hypothesis is that sex differences in lifespan are adaptive and driven by sex-specific survival-reproduction trade-offs. Due to differences in reproductive roles between the sexes, natural selection may favour different optima in life history strategies for each sex. In many species, females experience higher mortality costs from offspring production, whilst males can encounter trade-offs linked to mate competition. However, these verbal arguments are based primarily on empirical meta-analyses, lacking formal theoretical predictions. To address this, we developed the first theoretical model to investigate how sex-specific trade-offs shape lifespan evolution in a monogamous mating system. In our model, individuals evolve to optimise a trade-off between reproduction and survival, which operates through access to mating

opportunities in males and offspring production in females. By systematically varying the strength of these trade-offs, we find that either sex can evolve greater longevity, but males outlive females under a broader range of conditions, aligning with evidence from monogamous species. This challenges the common verbal argument that the sex competing for mates (often males) is likely to live shorter due to the survival costs of sexual selection. In our model, female longevity is more constrained than male longevity because the female trade-off between offspring production and survival directly affects the fertility of both sexes. In contrast, the male trade-off involving mate competition has only a weak, indirect effect on female fertility via mating dynamics. As a result, males outlive females under a broader range of conditions. These findings offer new theoretical insights into the adaptive bases of sex-biased longevity and highlight the importance of life history trade-offs in shaping lifespan evolution.



Connecting moth flight timing, ageing and predation risk – a citizen science approach

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POSTER SESSION 2 - P02.014, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

One of the objectives in life-history research is to understand how evolution has shaped current diversity in ageing rates. The historical ecological context plays an important role in explaining life-history diversity between and within species, often showing a relation between food abundance, predation risk and ageing.

Although the evolutionary connection between mortality risk and life-history evolution is well studied in the laboratory, studies in more ecological settings also have shown their significance in evolution and ecology shapes life-histories, but are still scarce.

Many bat- and nocturnal moth species are involved in an arms race, and in response to this predation risk moths often adapted different counter strategies resulting in a diversification of many traits, such as wing shape and wing patterning. However, also life-history diversity can be connected to predation risk within Lepidoptera but is poorly investigated.

Bats have been regarded as the most important predator for nocturnal moths. And the most abundant (Dutch) bat species seem to concentrate their flight activity around specific periods of the night, this may be a suitable system to study temporal patterns of predation and co-evolved life-histories. Although other (a)biotic factors could strongly co-vary with moth life-history, such

as temperature and food abundance, we also expect differential life-history adaptations to be evolved for different moth species that fly at different periods during the night.

Indeed, our first pilot studies seem to show time-dependent flight patterns of different moth species, and an inverse relationship between a species' body size, time-dependent flight and bat abundance. However, as this is early seasonal data, and therefore strongly biased on moth species more abundant in spring.

In this citizen science project we further investigate this relationship over multiple seasons. By using a light trap system that per night samples nocturnal moths only on specific time slots, the next morning visitors of a botanical garden can contribute to this project by determining the caught individuals. When performed multiple nights, locations, and time intervals, this citizen science data may contribute on determining species-specific flight patterns.

Additional measurements on ageing-related life-history characteristics, their phylogenetic distribution, and their relation to bat predation risk could show whether (certain taxa) of nocturnal moths can serve as potential new model system to study the ecological piece of the puzzle explaining diversity of ageing.



Environmental influences on sex-specific senescence in Soay sheep: insights from a longitudinal study

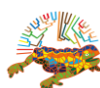
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POSTER SESSION 2 - **P02.010**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Sex is a major source of variation in senescence rates and sex differences in senescence can be seen in a range of taxa and across a range of traits. The energetic costs of reproduction are expected to be unequal between the sexes, with males, particularly those from polygynous populations, allocating more energy towards secondary sexual characteristics and sexual behaviour and resulting in higher mortality costs to males. However, a recent meta-analysis of over 100 wild mammals did not find any evidence of consistent sex-differences in ageing rates. In this work, we aimed to test whether sex-differences in the expression of senescence rates are dependent on environmental conditions. Although age-related susceptibility to extrinsic hazards is well-documented in humans, examples from natural populations are still rare. Despite predictions on how both sex and environmental hazards influence ageing rates, theory and empirical study of the combined consequences of these two factors on senescence are both limited and of mixed results. Using the long-running individual-based study on the Soay sheep of St Kilda, Scotland, we utilised almost 40 years of data to investigate the differences in survival and reproductive senescence between the sexes, and if these differences were dependent on population size. The Soay sheep are a sexually dimorphic, polygynous population of free-living sheep, known to face wide variation in environmental conditions. High population sizes, associated with increased competition for resources and increased parasite burdens, are known to influence over winter survival and male reproductive success. We predicted that the interaction between the costs of sexual selection and increased susceptibility to environmental conditions with age would lead

any differences in senescence rates between the sexes to be exacerbated under high population sizes. We tested the interactions between age, sex, population size, and their associations with annual survival, annual breeding probability, offspring birth weight and recruitment success of females, and annual offspring number of males. Although we found both sexes experienced decreasing survival probability with age, there was no difference between the sexes in the rate of survival senescence. Across the age range, high population sizes decreased survival probability, although there was no effect of population size on the rate of survival senescence or the lack of difference in survival senescence between the sexes. We also present here the first evidence of senescence in male reproductive success for this system. Both sexes showed significant rates of decline in annual breeding probability with age, although there was no difference between these slopes. Neither the senescent slope of males or females were influenced by population size, nor was there any association between population size and breeding probability. Although female recruitment success and offspring birth weight were both found to senesce, the rate of decline of neither trait were impacted by population size, although offspring birth weight was generally reduced under higher population sizes. Male annual offspring number was also reduced at higher population sizes, and the senescent rate of this trait was significantly faster at high population sizes. This work aims to fill a gap in ageing research, by investigating the causes of variation in senescence rates, and how the interactions between condition and the environment can influence the expression of senescence.



Epigenetic Clocks of Soay Sheep

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POSTER SESSION 2 - P02.008, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Ecologists studying wild animals are often interested in understanding the how and why individuals age, and whether ageing rates vary by individual. Recent technical advances in predicting ageing in humans and mice are now being applied to wild animals. First generation epigenetic clocks are used to estimate chronological age of an individual based on its methylome. The methylome is made up of CpG sites, some of which gain or lose a methyl group, through (de)methylation, at a constant rate through lifespan. The difference between this epigenetic age and actual chronological age can be used to estimate individual ageing rates. Second generation clocks, such as the human PhenoAge and GrimAge clocks incorporate phenotypic health and disease risk factors such as smoking pack years. These clocks can be used to assess directly predict biological age and potentially the time to death. Following this I am asking the question, can these first and second generation epigenetic clocks be built for a wild animal context, to measure chronological age, biological age, and to

predict time to death? The long-term data of the Soay Sheep of St Kilda provides the perfect study system to answer this question. Individuals have been sampled repeatedly throughout their lifetime, providing a wealth of longitudinal life history data, including phenotypes that show patterns of senescence. Based on the methylomes of around 1000 samples, I built a clock using elastic net regression, which selected the CpG sites that best predicted age. Having created a chronological clock, the next step is to build in markers of biological age. Identifying which phenotypes can together predict animal age and health will help pinpoint CpG markers of biological ageing. Phenotypic measures such as immunological assays, number of adult teeth, and parasite burdens may indicate both age/maturity and condition of an individual. Combining these with epigenetic markers could produce powerful clocks for both chronological and biological age to deepen our understanding of diverse trajectories of life in the wild.



Exploring the causes of lifespan differences among dog breeds: a burgeoning model for longevity research

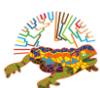
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POSTER SESSION 2 - P02.007, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Animals show substantial variation in lifespan, often associated with body size. Across species, larger animals tend to live longer than smaller ones. However, within species, larger individuals typically have shorter lifespans, both in wild and in domestic animals. This apparent contrast highlights the need to understand the factors driving lifespan variation across and within species. The domestic dog is a burgeoning model for longevity research: most extrinsic causes of mortality are controlled and breeds show extreme phenotypic variation, with a twenty-fold range in body size, and a two-fold difference in lifespan among breeds. Because extrinsic mortality is largely absent in dogs, a promising approach to understanding among-breed differences in lifespan is exploring whether there are breed-specific susceptibilities to different diseases. Previous work, for example, suggests that larger breeds are more susceptible to certain types of cancer. The evolutionary history of dogs also provides a valuable opportunity to analyze the influence of shared ancestry and the hybridization events that have given rise to modern breeds on disease susceptibility. First, we assessed whether variance in mortality due to different

diseases could be attributed to shared ancestry and hybridization events. Second, we analyzed the association between weight, growth, reproductive investment, and breed susceptibility to die from specific diseases, accounting for phylogenetic non-independence. We used published data from the Veterinary Medical Database (VMDB) combined with breed-specific data on adult size and life history. We found that the variance explained by shared ancestry and gene flow differed across disease types. Larger breeds were more likely to die from neoplasia, infectious diseases, and gastrointestinal, hematopoietic and musculoskeletal diseases. In contrast, smaller breeds were more prone to degenerative and urogenital causes of death. Greater reproductive investment was associated with reduced mortality from congenital, degenerative and inflammatory causes, independent of body size. These results highlight the role of the evolutionary history and the interplay between life history traits – specifically body size and reproductive investment, in shaping disease susceptibility and lifespan variation across domestic dog breeds.



Extreme Sexual Size Dimorphism: Sex-Specific Growth Pattern in a Spider

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POSTER SESSION 2 - **P02.020**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

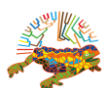
Female-biased sexual size dimorphism (SSD), where females grow significantly larger than males, is common in invertebrate species. While SSD has been widely studied from evolutionary and ecological perspectives, its proximate mechanisms remain poorly studied. However, understanding the developmental pathways in each sex, including differential growth patterns that give rise to SSD, is crucial to uncover the mechanistic basis of SSD.

Few species exhibit SSD as extremely as the African hermit spider, *Nephilengis cruentata*. Females are on average 75 times heavier and develop 2.3 times longer than males, which suggests that females may grow both, longer and at faster rate than males. Previous research indicates that variation in adult body mass is affected by hatching season. Adult body mass is primarily influenced by direct genetic effects in females and maternal effects in males. Although body mass is often used as a proxy for body size, it is more susceptible to short-term environmental influences than carapace width (CW), which grows in a determinate and discrete manner. As such, CW may provide a more developmentally stable and informative measure of body size.

In this study, we investigated the growth pattern—defined as the growth rate over developmental time—in *N. cruentata*, using CW as a proxy for body size. To compare growth patterns between sexes, we monitored 400 females and 1,203 males, measuring individual CW at least twice during development, from hatching to maturation. In addition to examining sex-specific growth trajectories, we also tested whether these patterns varied with hatching season.

In both sexes, CW increased with age in a non-linear fashion, but the growth pattern differed substantially between sexes. Both sexes initially exhibited a linear growth rate and did not differ in CW until approximately day 60, when males, upon reaching the subadult stage, began to slow their growth. In contrast, females continued growing at a similar rate until around day 200—when they reached the subadult stage—after which their growth also slowed. This implies that females grow bigger due to prolonged period of fast growth, not due to generally faster growth rate over development. At the average developmental age, females had CW of 1.198 mm (95% CI = [1.095, 1.311]) wider than males, and was significantly influenced by hatching season. However, while body mass at maturation was correlated with hatching season, adult CW was not, reinforcing its value as a more stable indicator of adult body size. Ultimately, adult females developed carapaces that were, on average, 3.76 times (95% CI = [3.23, 5.52]) wider than those of adult males.

Taken together, our findings offer novel insights into the developmental basis of extreme SSD in spiders. The detected sex-specific growth trajectories provide a foundation for future, more targeted investigations into the underlying molecular mechanisms—such as sex differences in gene expression and associated physiological changes—that likely emerge around the period when growth patterns begin to diverge between sexes. This offers a mechanistic link to the emergence of sexual size dimorphism. We also highlight the utility of carapace width as a robust trait for studying growth related to SSD in spiders.



Investigating the Plastic and Evolutionary Effects of Isocaloric Macronutrient Composition on Aging and Organismal Performance in Outbred *Drosophila melanogaster* Populations

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POSTER SESSION 2 - **P02.013**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Abstract: Aging is influenced by genetic, environmental, and metabolic factors, leading to diminished physiological functions and heightened disease risk. Nutrition, particularly the balance of proteins and carbohydrates, plays a significant role in this process. However, many studies have been conducted under ideal conditions, not reflecting the environmental stressors present in natural settings. This underscores the need to reassess nutritional interventions within varied environmental contexts. Additionally, some research conflates calorie intake with food composition, limiting applicability. While calorie restriction has shown benefits, it may lead to malnutrition and is impractical for many. Thus, adjusting food quality and macronutrient composition is a more feasible approach. Furthermore, nutritional needs also vary across different life stages, suggesting that stage-specific diets could optimize performance under various stress conditions. Exploring how these tailored diets influence organismal performance and their correlation with longevity remains a crucial area of research.

Materials and methods

I'm investigating the plasticity and long-term evolutionary effects of isocaloric dietary manipulation in outbred *Drosophila melanogaster* populations, varying protein-to-carbohydrate ratios at both larval and adult stages. To assess age-related organismal performance, experiments are conducted at early, mid, and late life stages. I've measured starvation, desiccation, thermal, cold, and osmotic tolerance to evaluate fitness under diverse environmental conditions. To explore the biochemical basis, I've quantified key nutritional reserves like triacylglycerides (TAG) and protein, and used the negative-geotaxis assay as a behavioral proxy for neuromuscular aging.

Results: Single-generation response

A high-protein larval and high-carbohydrate adult diet enhances early-age starvation resistance, while a high-carbohydrate adult diet improves it at mid and late ages. High-carbohydrate diets also boost desiccation resistance across most age points. Thermal tolerance is supported by both high-carb. larval and high-protein adult diets in early life, and by high-protein diets at mid-life. Osmotic stress resistance increases with a high-carb larval and high-protein adult diet. Biochemically, greater TAG and protein levels correlate with improved starvation resistance. Interestingly, flies raised on carbohydrate-rich diets during both larval and adult stages show better performance in the negative geotaxis assay.

Evolutionary response: Flies evolved under high carbohydrate food show better Starvation resistance in females, with no difference in males.

Our results underscore the effectiveness of stage-specific diets in enhancing performance across different stress contexts, offering insights into realistic dietary interventions that promote healthy aging and stress resilience.

Conclusions: We find the impact of both larval and adult nutrition on differential stress response for most of the assays. We also see an interaction between larval and adult nutrition, highlighting the importance of stage specificity and the complexity of nutritional requirements. Finally, the responses in some cases are different between males and females, thus emphasizing the sexually dimorphic nature of diet and its impact on aging and organismal performance. Interestingly, no particular diet improves all stress resilience, and the impact of diet is also age-dependent. Some stress responses correlate positively with longevity while others do not, thus highlighting the complex relationship between longevity and stress resilience.



Molecular and structural diversity of multi-ciliated cell types during larval development in the marine annelid *Platynereis dumerilii*

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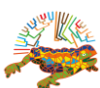
POSTER SESSION 2 - P02.003, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Multi-ciliated cells (MCCs) are specialized cells equipped with numerous motile cilia, essential for fluid propulsion and locomotion across a wide spectrum of organisms including vertebrates and invertebrates. Despite their importance, MCCs in marine invertebrates are still relatively understudied. The spiralian marine annelid *Platynereis dumerilii* serves a valuable model for studying ciliated structures during development, exhibiting distinct ciliary patterns and bands in its larval stages. This study aims to provide an in-depth analysis of MCCs in *P. dumerilii* larvae, focusing on their developmental stages, structural organization, and underlying gene expression dynamics.

Materials and methods: To do so we used immunostainings and whole mount in situ hybridization techniques, complemented by two ultrastructural whole-body cellular atlases for a three-day old free-swimming larva and six-day old settled juvenile worm, respectively. To investigate molecular diversity, single-nuclei RNA sequencing (snRNA-seq) was conducted across developmental stages (9, 12, 15, and 48 hpf).

Results: We manually traced 80 unique MCCs, classified into seven distinct subtypes by analyzing over 18,107 basal bodies and 14,069 cilia in one three-day-old *Platynereis* larva. These MCCs exhibited two major organizational patterns: (1) dispersed arrays of cilia in the prototroch, crescent cells, nuchal organs, and telotroch, and (2) aligned arrays of cilia as well as an antero-posterior polarity of basal bodies with and without cilia in para-, meta-, and akrotrachs. Transcriptional profiling through snRNA-seq revealed that early developmental stages (9 - 15 hpf) showed minimal differences in MCC gene expression profiles. However, by 48 hpf, distinct transcriptional signatures had emerged, indicating the establishment of transcriptionally specialized MCC populations. This study provides the first complete mapping and quantification of cilia in a marine invertebrate.

Conclusions: These findings offer valuable insights into the molecular differences between MCCs in ciliary bands of marine invertebrates, serving as an entry point for a molecular dissection of the cell biology of dispersed versus aligned MCCs and contribute to a deeper understanding of cell type changes over evolutionary time scales.



Nonhuman primate species share Alzheimer-like neuropathology with humans

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POSTER SESSION 2 - P02.018, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

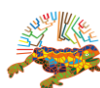
The hallmark neuropathologies of Alzheimer's disease (AD) include mature plaques (dense core and neuritic A β plaques), neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau, and cerebral amyloid angiopathy (CAA). Although AD-like neuropathology has been reported in several mammalian species, including domestic dogs and chimpanzees, a comprehensive, phylogenetically contextualised review of these pathologies across nonhuman primates, and broader mammals, remains lacking. This is especially important to investigate in nonhuman primates as they are our closest living relatives.

Following PRISMA (2020) guidelines, we conducted a systematic PubMed literature review targeting reports of A β and tau pathology in 295 nonhuman primate species. Observational and control animal data from peer-reviewed studies were included, while review articles, studies of structural brain changes without pathology assessment, and transgenic or experimentally-induced models were excluded. We collected the data on the presence, distribution, and age of onset of A β and tau pathology types. We aimed to characterise species-specific variation in the occurrence and distribution of AD-like neuropathologies, including

mature plaques, CAA, and NFTs, across nonhuman primates. In doing so, we inferred ancestral states and assessed the presence of phylogenetic signals underlying susceptibility to these pathologies.

To provide a broader phylogenetic context for primate evolution, we expanded our dataset to include 22 diverse non-primate mammalian species alongside primates. In our preliminary analyses, ancestral state reconstructions were performed separately for each pathology type. Across the species sample, the occurrence of CAA, mature plaques and NFTs exhibited a significant phylogenetic signal, suggesting heritable vulnerability among closely related species.

These findings suggest that the development of AD-like neuropathology not only in primates but across mammals is not random, potentially reflecting shared evolutionary or genetic mechanisms underlying AD-like pathology. Furthermore, this dataset enables investigation into patterns of co-occurrence and divergence of Alzheimer-like pathologies, highlighting how plaques, tangles, and vascular pathology are associated and distributed across primate species.



Nutritional trade-offs in model and non-model insects and its consequences for lifespan, reproduction and sexual conflict

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POSTER SESSION 2 - P02.004, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The evolutionary puzzle of aging is often approached through the lens of a few model organisms, yet emerging evidence reveals diverse senescence patterns across the tree of life. Here, I will present two important insights about the interplay between nutrition, lifespan, and fitness. First, I used *Drosophila melanogaster* as a platform for mapping multidimensional nutritional trade-offs across traits, life stages, and sexes. Combining insights from the Geometric Framework of Nutrition, I demonstrate how nutrient balance—not just quantity—can shape nutritional trade-offs between lifespan and all other reproductive and developmental traits. Specifically, my results uncovered clear evidence of nutritional trade-offs, where protein-to-carbohydrate (P:C) ratios simultaneously maximise lifespan also minimise reproduction.

Second, I developed a semi-automated algorithm which allowed me to broaden the perspective across nine insect species using a novel method for reconstructing optimal

nutrient landscapes from published data. The analysis uncovered a moderate but statistically significant phylogenetic signal in lifespan-maximizing diets, implying evolutionary conservation in nutrient-longevity relationships despite ecological and taxonomic diversity. Crucially, sex-specific dietary optima also varied phylogenetically, hinting at deep evolutionary roots of sexual conflict over optimum nutrition. The consequences of such intragenomic conflict remains to be properly investigated, and I provide some urgent questions that need to be uncovered by theoretical and empirical work.

Together, these findings across model and non-model organisms reposition nutrition as a central, but underexplored, determinant of lifespan and aging. This work contributes to a broader, comparative framework for nutritional research related to lifespan which is grounded in relevant ecological and evolutionary contexts.



Surviving on limited resources: effects of caloric restriction on growth, gene expression and gut microbiota in a species with male pregnancy (*Hippocampus erectus*).

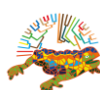
Pappert F¹

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POSTER SESSION 2 - **P02.001**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Caloric restriction (CR) studies have traditionally focused on species with conventional reproductive roles, emphasizing female's greater investment in costly gametes and parental care. While the divergent impact of CR on males and females is evident across species, the factors driving this variation, i.e., resource allocation to reproductive elements as part of distinct life history strategies, remain unclear. To address this, we investigated the effects of CR on development, gene expression, and intestinal microbiota in the lined seahorse *Hippocampus erectus*, a species with male pregnancy, where fathers invest in offspring through gestation. Juvenile seahorses were subjected to ad libitum (AL) or CR feeding for 5 months. CR stunted male growth and brood pouch development, reflecting the energy demands of this crucial parental care trait. However, condition index declined in CR females but not males, while ovarian

weight remained unchanged. Transcriptome analysis demonstrated organ- and sex-specific responses to CR with distinct lipid and energy-related pathways activated in male and female livers, indicative of survival enhancement strategies. CR had minimal impact on genes associated with spermatogenesis, but downregulated lipid metabolic and inflammatory genes in ovaries, emphasizing the importance of pre-copulatory resource allocation in female gametes. CR strongly shaped gut microbial composition, creating distinct communities from AL seahorses while also driving sex-specific taxonomic differences. Our research indicates that nutrient limitation's impact on males and females is influenced by their allocation of resources to reproduction and parental investment. We underscore the significance of studying species with diverse reproductive strategies, sex roles, and life-history strategies.



The ageing methylome of the King penguin

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POSTER SESSION 2 - **P02.017**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Understanding global molecular processes related to senescence is essential to unravel how fitness decays with age at a population level. Still, due to difficulties in obtaining large-scale samples and age-related information for most non-model species, our current knowledge about such aging processes in wildlife are limited. One of the best characterised ageing hallmarks is the methylation of cytosines followed by a guanine nucleotide, also known as CpG methylation. Although ageing methylation patterns are well characterised in mammals, these patterns are not always conserved in other vertebrate clades. As an example, key metabolic pathways, such as thermogenesis, are known to have evolved independently in birds, making the generalisation of ageing processes between clades likely erroneous. We explored the ageing methylome of the king penguin (*Aptenodytes patagonicus*), a long-lived seabird with an average lifespan of 20 years-old in the wild, in order to identify key genomic features and pathways related with the ageing process in the species. For those means, we sequenced 96 whole-blood methylomes from males and females of known age, sampled at different time points during their life. After fitting a generalised linear mixed-effects model to the individual CpG methylation frequencies, by setting age and sex as fixed effects, we detected 125,545 CpGs that were correlated with age in autosomes (age odds P-values < 0.01). The majority of age-correlated CpG methylated loci were located in intronic regions, followed by intergenic regions. Among

intronic CpGs, we observed an enrichment in the first intron, hinting towards an importance of the first intron in the senescence process of the species. First intron methylation is suggested to cause gene expression silencing in different vertebrate species. The top CpG related with age in a first intron was located in gene SH3RF3, which is upregulated in human cancer stem-like cells, and could be related to the silencing of tumour genes in younger individuals. Among our top hits located in other introns and exons, we detected CpGs inside gene ARNT2, a transcription factor related to development and responses to environmental stimuli; gene CDKN2C, related to cell growth; and, curiously, a peak of CpGs showing high variance with age was located in the METRNL gene, which is related to brown fat cell differentiation. METRNL is known for its role in adipose tissue browning and insulin sensitivity in mammals, and could become a key biomarker for birds if we consolidate its role in the ageing processes in our target species, but also other birds. Moreover, further investigation of gene expression patterns coupled with DNA methylation should shed light into the actual effects of methylation at the detected loci. Finally, most CpGs correlated with age were located farther than 2000 bp up- and downstream from a gene (i.e., gene deserts), not overlapping with non-coding elements currently annotated in the king penguin genome. These results demonstrate that exclusively focusing on CpGs inside genes may provide us with only parts of the potential patterns related with senescence.



The Mammal That Rewired Insulin Signaling to Live Long and Prosper on an Extreme High-Sugar Diet

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POSTER SESSION 2 - P02.021, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

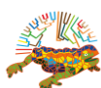
Nectar-feeding bats thrive on a diet composed almost entirely of simple sugars, sustaining blood glucose levels exceeding 750 mg/dL—levels that typically induce metabolic disease in other mammals. Yet, these bats live 10–15 years, similar to related species with balanced diets. To investigate this metabolic resilience, we analyzed postprandial gene expression across seven bat species and performed functional assays in primary muscle cells from three species.

Despite intact insulin production, nectar bat skeletal muscle shows insulin resistance, marked by the absence of AKT phosphorylation and glycogen storage. Instead, their muscle cells exhibit spontaneous contractile activity, suggesting enhanced AMPK activation—a key energy sensor that promotes fat oxidation and ketone production. Supporting this, nectar bats elevate circulating ketone levels even

during hyperglycemia, indicating a reliance on lipid metabolism at rest.

Increased lactate levels further suggest a glycolytic shunt via MAPK signaling, diverting glucose away from the TCA cycle. We propose that maintaining high blood glucose provides a circulating fuel reserve, sparing glucose for the extreme energy demands of hovering flight while prioritizing lipid-based metabolism at rest. Additionally, suppressed AKT signaling may activate FOXO3a, a transcription factor linked to stress resistance and longevity.

Together, these findings reveal that nectar bats have rewired insulin signaling to decouple longevity from the harmful effects of chronic sugar intake. This metabolic strategy prioritizes lipid oxidation, energy sensing, and muscle excitability, offering new insights into ageing and metabolic flexibility in mammals.



The relationship between phylogeny and age alignments in mammals

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POSTER SESSION 2 - **P02.019**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Different species develop and age at distinct rates and along divergent trajectories, posing a major challenge for comparative studies. Translating Time is a framework developed to align developmental stages across species. Our online tool, translatingtime.org, implements a model based on a dataset of neurobiological developmental events across diverse mammalian taxa. More recent work by our team has begun to extend this framework to support age alignment across the entire lifespan. In our multispecies models, time points in different species are averaged to create an event scale that is standardized across species spanning from conception (0) to maximum lifespan (1).

Currently, our database includes over 34,000 data points, representing 1,745 unique time points across 2,263 species. Translating ages across species is essential when comparing biological data types that vary over the life course. For example, transcriptomic profiles shift across stages of development and aging since different early gene expression patterns are critical for early tissue formation, and later, there are changes associated with age-associated gene regulatory decline. Age alignments are necessary to calibrate interspecies comparisons of transcriptomic profiles across tissues and cell types.

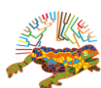
A major challenge is the lack of homologous time point data across biodiverse species. Some species have been studied intensively from experimental periods starting from conception, while others are only observed postnatally in the wild, often not until they emerge from a nest or burrow. This inconsistency results in missing data. Our framework addresses this using imputation and predictive modeling. However, we cannot extend alignments reliably beyond the age ranges for which robust, homologous data exist. Yet comparative analyses often require age translations at life stages where the timing of biological processes is insufficiently known to determine equivalency with confidence.

To address this limitation, we tested whether phylogenetic information could inform age alignments. Our preliminary analysis compared “hypothetical age equivalencies” which are models that include, but extend beyond, age alignment time points across 62 species. We found a moderate but statistically significant correlation between phylogenetic distance and divergence in trajectories, as measured by Euclidean distance between fitted polynomial curves. This suggests that more distantly related mammals exhibit more divergent life history trajectories, spanning from early development through aging.

We investigated the presence of phylogenetic signal at two time points: birth and death. Species were placed along an altricial-precocial spectrum by calculating the residual of the age in post-conception days at which birth occurs, to determine deviations from the multi-species model prediction. Thus operationalized, placement along the altricial-precocial spectrum was tested for phylogenetic signal. After an initial analysis showed phylogenetic signal, we tested alternative evolutionary models and found that a multiple-regime Ornstein-Uhlenbeck model, with separate adaptive peaks for altricial and precocial species, best explained the data. This suggests that these altricial and precocial species are evolving toward different, regime-specific optima.

We also calculated a residual of maximum longevity, expressed as a percentage deviation from the expected value predicted by the multi-species model. This could be used to identify species with exceptional lifespans to select as model organisms in aging research. An initial analysis revealed phylogenetic signal suggesting evolutionary conservation of the trait.

Our preliminary results shed light on the potential of phylogenetic comparative methods to inform about the evolution of life histories, support age alignment across species, and guide the selection of appropriate model organisms.



The role of selection pressure in the evolution of asynchrony of aging

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POSTER SESSION 2 - P02.002, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Ageing, the age-related changes in a trait, is a highly variable process. Studies have investigated variation in ageing among species and individuals. Different traits within-individuals may age differently, yet little is understood about why they differ. Historic theories predicting that ageing should be synchronous across traits are erroneous. Recent theories predict that the rate and onset of trait ageing should be explained by the selection pressure on the trait. To date, there have been no empirical studies examining the role of trait specific selection behind the evolution of asynchrony of ageing. First, we tested for asynchrony of ageing across 13 traits (including a range of morphological, physiological, behavioural and life history traits), in a closed natural population of Seychelles warblers (*Acrocephalus Seychellensis*). Secondly, we examined whether selection on a trait explained their respective ageing trajectories. We quantified synchrony between traits with similar ageing trajectories by comparing the onset and rate of ageing. Out of the 13 traits, 6 exhibited

ageing: body mass, fat score, provisioning rate, telomere length, survival and annual reproductive success, while buffy coat only showed aging in females. There was no support for synchronous ageing among traits although some traits shared similar aging trajectories. Our study adds to growing evidence that ageing is asynchronous. Our preliminary finding of ageing in traits expected to have stronger selection: e.g. survival and reproductive success, but not in a trait expected to have weaker selection e.g. wing length seems to indicate some weak support for newer theories that traits under stronger selection experience more ageing. We will next quantify selection on traits with significant ageing and test the hypothesis that traits with stronger selection should have more rapid and an earlier onset of ageing. We will present the results and discuss the implications of the study in the presentation. To our knowledge, this study will be the first empirical study to demonstrate the role of selection pressure behind the evolution of asynchrony of aging.



Aging in ants: from the individual to the colony level

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MEETING ROOM 129+130, August 19, 2025, 2:00 PM - 5:00 PM

Some species exhibit indeterminate growth, growing continuously with an increase in fertility long after maturity. For example, ant colony size continues growing after the queen's maturation – beginning of sexual production – and ant queens increase the production of sexuals with age. Two features of the ant *Cardiocondyla obscurior* imply that the strength of selection against senescence is maintained after the queen's maturity. (1) The mortality rate of ant queens is low compared to their average mortality past their fertility peak, with mortality only increasing rapidly very late in life. (2) There is no evidence for the Lansing effect, even if interpreted sensu lato as a negative effect on offspring fitness of advanced maternal age. In our project, we look beyond the individual to understand how colonies are established, mature, and age. New colonies are produced

by budding, where some individuals leave the mother colony on foot to establish a new daughter colony. How the superorganism ages will depend on the maturation time too, i.e., funding of daughter colonies by budding, and the allocation of individuals into new versus old colonies. Analogous to yeast, in which asymmetric budding produces a cell with rejuvenated genetic material and older with the accumulated DNA damage, we aim to know if new colonies were formed by old queens or young queens. Lastly, we experimentally varied the extrinsic mortality experienced by colonies to investigate whether colonies support theoretical predictions regarding colony maturation time. Our study sheds light on the understanding of senescence in organisms in which size affects mortality risk and other demographic determinants of aging.



Divergence in allelic variation and gene expression in molecular networks that underly aging between fast- and slow-aging garter snakes.

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MEETING ROOM 129+130, August 19, 2025, 2:00 PM - 5:00 PM

Background. Understanding the genetic underpinnings of natural variation in rates of aging can identify mechanisms for how such variation evolves. The process of organismal aging has been explained by many cellular networks in laboratory model organisms. Much less is known about whether these networks are conserved and causative in wild populations. A thorough understanding of the mechanisms of aging requires examination of patterns in wild populations, since this is where aging evolved. Here we use a long-studied system of garter snakes (*Thamnophis elegans*) in the Eagle Lake basin (Lassen County), California US that has replicate populations characterized by two life history strategies of fast-aging (faster growth to larger body size, more frequent reproduction, and shorter lifespan) and slow-aging ecotypes. Previous research found phenotypic divergence between ecotypes in body size, color pattern, immune function, and age-related cellular signaling pathways. Here we test whether there are genetic underpinnings for these divergent ecotypes by examining variation and expression in gene networks within several major hallmarks of aging: Metabolic Function (including Insulin and Insulin-like Signaling [IIS] and Oxidative Phosphorylation), Macromolecule Damage and Repair, and Stress Adaptation.

Materials and Methods. We assess levels of genetic divergence among populations of the two ecotypes in (1) hepatic transcriptomic responses to acute heat stress using RNAseq, and (2) sequence variation from whole genomic sequencing data and targeted sequence capture data for putative aging loci through genetic distance (FST and DXY), and amino acid polymorphisms between fast- and slow-aging snakes.

Results. Through differential gene expression and pathway enrichment analyses we find significant divergence between the fast- and slow-aging ecotypes in their response to heat stress,

specifically in their regulation of the IIS, Oxidative Phosphorylation, and DNA repair molecular networks. The slow-aging ecotype had individuals with greater coordinated transcriptomic response to heat stress relative to fast-aging, which may result in the coevolutionary process for stress-resistance and senescence-resistance. At the level of sequence variation, we find allelic divergence in key nodes in these networks. At the intersection of divergence in gene expression and divergence in sequence variation, we find many Insulin-Signaling genes (including IGF2, and IGFBP3, IRS4, GSK3B, MAP3K1, and FOXO3), many Heat Shock Proteins, and DNA repair genes (including XRCC3 and RAD54B) that have diverged in gene expression and sequence variation. Many of the genes and pathways identified as divergent among our fast- and slow-living ecotypes have been associated with longevity in several long-lived organisms.

Conclusions. Using natural populations of garter snakes that have diverged in their physiology and life history strategies, we have documented putative mechanisms through divergence in regulation and nucleotide sequence of candidate gene networks underpinning three hallmarks of aging: metabolic processes, macromolecule damage and repair, and stress adaptation. In doing so, we have demonstrated these candidate molecular networks regulating aging translate from lab models to natural populations. In addition, the interaction between ecotypes in response to heat stress indicates many more pathways are diverging than just the ones we highlighted in this study, suggesting natural populations and non-model organisms likely have mechanisms for diversification in aging processes beyond those currently identified in lab models. This research provides insight into our understanding of how life history traits, including aging, evolve, and highlights the importance of studying cellular networks of aging within and across species.



Germline mutation rate and somatic maintenance improved simultaneously by reduced insulin signalling

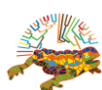
Duxbury E¹, Godden A¹, de Coriolis J¹, Carlsson H¹, Immler S¹, Maklakov A¹

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MEETING ROOM 129+130, August 19, 2025, 2:00 PM - 5:00 PM

Disposable soma theory maintains that organisms age due to trade-offs between an immortal germline and a disposable soma. Yet increasing evidence demonstrates that lifespan extension is not always accompanied by a fecundity cost. One of the most compelling examples of apparent cost-free lifespan extension is the downregulation of adulthood insulin/IGF-1 signalling (IIS) in *C. elegans* nematodes, using *daf-2* RNA interference (RNAi) knockdown in adults, making them extremely long-lived and stress-resistant with normal reproduction. We tested whether this apparent absence of a trade-off between survival and reproduction, can be explained by the hidden costs of reduced germline maintenance, leading to a trade-off between parental longevity and offspring fitness. We ran a multigenerational mutation accumulation (MA) experiment to directly compare germline mutation rates in replicate *daf-2*

RNAi and control MA lines after inducing germline mutations using UV irradiation. We tested whether increased investment into somatic maintenance via reduced IIS resulted in increased mutation rate. Contrary to predictions, we found reducing IIS results in reduced germline mutation rate. Using high coverage whole genome sequencing of the MA lines, we showed reduced rates of germline point mutations and transposable element insertions under reduced IIS in UV-irradiated MA lines, with fewer missense mutations in genes with essential functions in development or reproduction. Interestingly, functional inheritance of RNAi was required for the benefits of multigenerational *daf-2* RNAi. Our results suggest that reduced insulin signalling improves both somatic and germline maintenance. We discuss evolutionary implications of these findings.



Lifespan extension in *Heliconius* butterflies: from ultimate to proximate mechanisms

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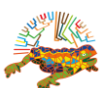
¹University Of Bristol

MEETING ROOM 129+130, August 19, 2025, 2:00 PM - 5:00 PM

The diversity of lifespans across the animal kingdom has long been a subject of interest for evolutionary biologists. With maximum longevities ranging from a single day in adult mayflies up to several centuries in the ocean quahog, how have some organisms managed to evolve such long lives? With a globally ageing population, this question is of growing biomedical interest – and the high conservation of mechanisms of ageing has researchers increasingly looking to non-model systems for the answers.

The remarkable lifespans of the *Heliconius* butterfly genus, stretching up to almost a year, rank among the longest recorded in butterflies. Crucially, this lifespan extension has occurred relatively recently in evolutionary time, with recorded lifespans of only 1-2 months for their nearest relatives in the *Heliconiini* tribe. This longevity has been

linked to the evolution of a unique pollen-feeding behaviour in *Heliconius*, which co-occurs with a suite of other behavioural and physiological traits that are absent in the non-pollen-feeding *Heliconiini* outgroups. Here, we combine survival analyses, behavioural assays, and transcriptomic work across the lifespan of *Heliconius hecale* and the shorter-lived *Heliconiini* outgroup *Dryas iulia*, under both pollen-fed and pollen-deprived diets. This approach allows us to construct an exhaustive profile of ageing in these butterflies, shedding light on the evolutionary trajectory of *Heliconius*' lifespan extension and some of the key molecular mechanisms that underpin it. Our results help to disentangle the link between diet and ageing in *Heliconius*, adding a new case study to the canon of noteworthy agers, and providing valuable insights into the evolution of long life.



Peto's Paradox for fungi

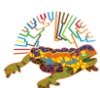
Aanen D¹, **Auxier B**¹

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MEETING ROOM 129+130, August 19, 2025, 2:00 PM - 5:00 PM

Peto's paradox refers to the absence of a correlation between body size and cancer risk in animal species. The solution of this paradox comes from the realisation that natural selection interacts with the life history of a species and favours adaptations that suppress cancer through the expected period of fertility of an organism. So tall -and typically long-lived- species must have robust cancer-suppressing mechanisms to achieve a low lifetime cancer risk, while small -and typically short-lived- species can do without, to achieve the same low lifetime-cancer risk. It seems impossible to apply this logic to modular organisms, such as fungi, since they do not have a fixed body plan and thus can continue to increase in size. Moreover, what are cancerous mutations in modular organisms, which do not have an early separation between soma and germline? I propose an analogue of Peto's paradox for fungi, based on the following experiments, observations and logic: i) The mycelial lifespan of fungi varies extensively between species and is -at least partially- determined by extrinsic factors such as fire, finite and discrete growth substrates or

ecological strategy; ii) Using experimental evolution, we demonstrated that short-lived fungi predictably evolve cheating variants during clonal propagation. Those cheating variants are analogous to cancer mutants in animals, since they have a competitive benefit within the fungal mycelium, but reduce mycelium-level fitness; iii) In contrast, naturally long-lived species, such as the fairy-ring fungus and the honey fungus, are resistant to the evolution of cheating variants and so far, experimental evolution has failed to show the selection of cheating variants; iv) Consistent with the resistance of long-lived species against cheating mutants, those species typically have mechanisms that we hypothesised reduce the selective scope of selfish mutants, such as a low mitotic mutation rate, a higher degree of cell compartmentalisation, and clamp connections. Analogous to Peto's paradox, we thus propose a constant and low lifetime cheating risk for fungi, which explains the low natural occurrence of cheating mutants in the kingdom Fungi, which contains species that vary in life expectancy from days to millennia.



Senescence evolution under the catastrophic accumulation of deleterious mutations

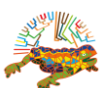
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MEETING ROOM 129+130, August 19, 2025, 2:00 PM - 5:00 PM

For aging to evolve, selection against mortality must decrease with age. This prevailing view in the evolutionary theory of senescence posits that mutations with deleterious effects happening late in life—when purging selection is weak—may become fixed via genetic drift in the germline, and produce a senescent phenotype. Theory, however, has focused primarily on growing populations and the fate of single deleterious mutations. In a mathematical model, we demonstrate that relaxing both of these simplifying assumptions leads to unrealistic outcomes. In density-regulated populations, previously fixed deleterious mutations should promote the fixation of other deleterious mutations that lead to senescence at ever younger ages, until death necessarily occurs at sexual maturity. This sequential fixation of deleterious mutations is not promoted by a decrease in population size, but is due to a change in the strength of selection. In an individual-based model, we

also show that such evolutionary dynamics should lead to the extinction of most populations. Our models therefore make rather unrealistic predictions, underlining the need for a reappraisal of current theories. In this respect, we have further assumed in our models that the deleterious effects of mutations can only occur at certain ages, marked, for instance, by somatic or physiological changes. Under this condition, we show that the catastrophic accumulation of deleterious mutations in the germline can stop. This new finding emphasizes the importance of investigating somatic factors, as well as other mechanisms underlying the deleterious effects of mutations, to understand senescence evolution. More generally, our model therefore establishes that patterns of senescence in nature depend not only on the decrease in selection strength with age but also on any mechanism that stops the catastrophic accumulation of mutations.



Senescence in an unusual invertebrate model: insights from the tsetse fly

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MEETING ROOM 129+130, August 19, 2025, 2:00 PM - 5:00 PM

Here, I show what insights we can gain on understanding the evolution of senescence by considering a viviparous insect with high maternal investment - the tsetse fly - as an unusual model. While tsetse are unitary and sexually reproducing, they contrast typical systems to study survival and reproductive senescence due to their viviparous reproduction (mothers give birth to single offspring the same size as the parent,

iteroparously across their relatively long life) and role as vector of trypanosome parasites. Here, I show how what we have learned about survival and reproductive senescence using a combination of theory, lab and field studies in tsetse, and the implications of this research both for testing predictions about the evolution of ageing and for understanding its role in in epidemiological models of tsetse-borne disease.



The antagonistic pleiotropy and disposable soma theories of ageing differ in their predictions of age specific survival and fecundity

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MEETING ROOM 129+130, August 19, 2025, 2:00 PM - 5:00 PM

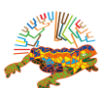
A main question in evolutionary biology in general and ageing research specifically, is how genetic variation translates into phenotypic variation. Furthermore, whether new mutations affect lifespan and other traits solely negatively, via mutation accumulation, or through trade-offs remains largely undetermined. Trade-offs are expected to affect certain traits negatively, while others are altered positively. In the history of evolutionary theories of ageing, two theories have resided next to each other that consider trade-offs as a basic mechanism to describe ageing.

These two classical evolutionary theories of ageing - antagonistic pleiotropy (AP) and disposable soma (DS) theory - are based on trade-offs at different biological levels (genetics and physiology) but many predictions of these theories overlap. However, a key difference between AP and DS lies in when a new mutation negatively affects survival, compared to its positive effects on other traits. AP assumes that negative effects are expressed relatively late in life compared to positive effects on for instance fecundity, while DS assumes that an increase in fecundity directly has negative consequences survival.

We use a life history theory model to test whether this difference in timing of these

effects results in different predictions for life courses, specifically age-dependent survival but also fecundity. We find that AP predicts a threshold age for these traits, such that fecundity and survival for age classes below this threshold are maximized while no individuals survive beyond this threshold age. On the other hand, DS predicts selection of mutations that cause a monotonous increase in mortality, which leads to survival curves that are seen in real life. Furthermore, in simulations that contain only a small proportion of AP, compared to DS mutations, the resulting life course predictions are equal to AP predictions.

We conclude that if in our world trade-offs are based solely or partially AP mutations, survival curves would look very different from the ones found on planet earth. Hence, genetic effects that have previously been attributed to AP are likely of the DS type. This means that (negative) phenotypic effects that are only observed late in life must have a physiological basis early in life. These findings have important implications for how age-dependent mutations affect fitness-related phenotypes, such as fecundity and survival, and should therefore be carefully considered when generating and interpreting empirical data.



The selection shadow measured at the single-cell type level

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MEETING ROOM 129+130, August 19, 2025, 2:00 PM - 5:00 PM

In recent years various studies have shown that genes expressed at old age (old-biased genes) evolve under weaker purifying selection than young-biased genes. However, these results were based on transcriptome profiles of either bulk tissue samples (from primates and rodents) or whole-body samples (of Diptera). It has thus been unclear whether this "selection shadow" signal might be simply caused by tissue and cell type composition changes over life, and also whether it might be observed across a wider range of species. Here we first confirm that the signal of weak purifying selection on old-biased genes can be observed across brain and/or liver bulk tissue transcriptomes from a wide range of

metazoa, from the naked mole rat to chicken to fruit flies. We then show that the signal can also be observed in single cell type transcriptomes from mice. Although different cell type transcriptomes appear to be under diverse levels of overall purifying selection, comparing their profiles in young and old animals, the majority demonstrate the selection shadow effect. This marks that the observed signal is not caused by cell type composition changes. We discuss how genetic drift on regulatory and/or coding sequences could be a widespread contributor to ageing phenotypes and how species with negative senescence would be used to gauge these potential contributions.





ESEB2025
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**CONGRESS OF THE EUROPEAN SOCIETY FOR
EVOLUTIONARY BIOLOGY**

**S05 - Aliens among us: ecological drivers,
evolutionary dynamics, and rapid
ecosystem reshaping by biological
Invasions**



Bottlenecked but booming: paradoxically strong inbreeding in highly invasive freshwater mussels

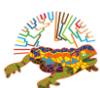
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POSTER SESSION 1 - P01.063, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Biological invasions threaten global biodiversity and human well-being, which highlights our pressing need to better comprehend still poorly understood invasion processes. Specifically, invasive populations originating from only few founders are expected to exhibit reduced genetic diversity and increased inbreeding. Theory predicts this should often lead to inbreeding depression and restrict adaptation in the invaders. However, successful invasions do often involve severe bottlenecks and founder events. We address this “genetic paradox of invasions” by analysing whole-genome resequencing data of invasive populations of the zebra mussel (*Dreissena polymorpha*). We provide evidence for extremely strong inbreeding in these highly successful invasive populations. We demonstrate that genomes

of the invasive freshwater mussels harbour extensive runs of homozygosity resulting from severe bottlenecks and subsequent inbreeding during the invasion process. We also quantify deleterious genetic variation (genetic load) across populations from the native and invasive range of the species and find indications of the possible role of purifying selection (purging) in facilitating invasion success in these mussels. This study provides a compelling example of a paradoxically successful invader and offers novel insights into the genomic consequences of inbreeding due to founder events and bottlenecks in invasive species. These findings are broadly relevant across taxa and contribute to a deeper understanding of factors affecting invasion dynamics.



Coexistence and Succession in Disequilibrium: Insights from Nematode Communities on Decaying Beetles

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POSTER SESSION 1 - P01.055, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

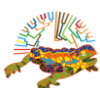
Background: Understanding the processes that shape ecological communities and determine their dynamics in relation to other such communities arranged in space - i.e., a metacommunity - remains central to ecology. The majority of theoretical treatments of this topic rely on models suited to study ecosystems at equilibrium. In contrast, many ecosystems are transient, most notably those that emerge following environmental disturbance, which paves the way for species succession. Despite their long-appreciated importance, such transient ecosystems remain poorly understood.

Materials and methods: In this contribution, I present a novel approach to study the ecological dynamics on the carcass of the *Gymnogaster* beetle, as a model for transient, decaying ecosystems. Following the natural death of a beetle (environmental disturbance), a new habitat is created in which bacteria and fungi decompose the carcass. This creates resources for nematodes — hitherto passive hitchhikers on the beetle — to exit their dormant state in succession, consume bacterial resources, and beget new dormant larvae that migrate into the environment. This life cycle provides a perfect model to address the factors that shape metacommunity composition

consisting of transient communities formed by succession.

Results: We sacrificed *Gymnogaster* beetles sampled from Réunion Island in the laboratory and followed the emergence of nematodes on the carcass. The nematode species that emerged on these beetles exhibited diverse reproductive modes - hermaphroditism and gonochorism - as well as morphological variation in feeding structures. Some nematodes are obligate bacterial feeders, while others exhibit intraguild predation by preying on competing nematodes. To study the dynamics of coexistence in disequilibrium, we computationally reconstructed decaying beetles using an agent-based model, taking advantage of experimentally-derived life-history parameters for each species, i.e. emergence time, sex ratio and fecundity.

Conclusions: Our integrated analysis shows coexistence in transient ecosystems shaped by succession strategies. This work addresses the shortcomings of classical approaches and highlights the importance of transient interactions in ecosystems characterized by disequilibrium. Thus, our work provides a unique experimental and computational framework to synthesize various approaches to metacommunity and species succession theories.



Contrasting genomic signatures of hybridisation across admixed populations of native and invasive *Mytilus* mussels in Australia

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POSTER SESSION 1 - **P01.062**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

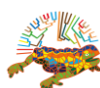
Background: Species are facing increasing pressure to rapidly adapt in the face of anthropogenic change. Introduced species are frequently used to study rapid adaptation to novel environments and can impose strong selection pressures on native congeners. Hybridisation between native and invasive species can facilitate rapid genetic change in both species by reshuffling the genome to unlock new adaptive combinations or transfer adaptive variation from one species to the other. Studying the impacts of hybridisation on native and invasive genomes can provide insights into how invasive species reshape native genetic diversity and how these new mosaic genomes may respond to selection and facilitate invasive spread. In Australia, two introduced lineages of the European invader, *Mytilus galloprovincialis* (Atlantic and Mediterranean) hybridise with the native mussel *Mytilus planulatus* forming replicated hybrid zones.

Materials and Methods: Here, we characterise hundreds of mussels using a

SNP genotyping panel to examine patterns of hybridisation along the genome. Contrasting these two hybrid zones, we use local ancestry inference, and scans for diversity and differentiation to test if selection has influenced introgression patterns differently in different genetic backgrounds.

Results: We find that admixture is extensive and parental European genotypes are not found. However, the two independent hybrid zones are characterised by contrasting major parent ancestries and locus specific patterns of genetic variation suggesting diverse outcomes of hybridisation among closely related taxa.

Conclusions: These different outcomes indicate hybridisation is context dependent and selection may be acting differently with different invasive genetic backgrounds. The Australian *Mytilus* system suggests the potential for multiple pathways of successful rapid adaptation following hybridisation of invasive species with native congeners.



Evolution of the genetic load in invasive insects: insights from a genomic cross-species study

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¹INRAE

POSTER SESSION 1 - P01.053, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

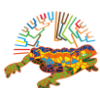
Background: Biological invasions are a major component of global change. Yet it remains unclear why some introduced populations become invasive while others do not. One intriguing hypothesis is the purging of deleterious mutations during the introduction process. Following the introduction of a small number of individuals into a new area, the interplay between genetic drift, inbreeding and natural selection may lead either to the fixation or to the purging of deleterious alleles, affecting individual fitness. We hypothesize that populations that successfully become invasive are those that have purged part of their genetic load.

Materials and Methods: We investigate the purge hypothesis across a broad taxonomic scale, analyzing ten insect species with well-documented invasion histories. For each species, we sampled between five and ten key native and invasive populations, representative of major steps in their invasion routes. We performed whole-genome pool-sequencing and developed a reproducible analysis pipeline to identify, annotate and categorize hundreds of thousands of SNPs per species. This framework enables a quantitative comparison of genetic load between native

and invasive populations, and between species.

Results: As expected, genetic diversity is generally lower in invasive populations compared to native ones, reflecting historical bottlenecks associated with introductions. However, we observe strong interspecific variation in the severity of bottlenecks and the magnitude of genetic load. Across species, no consistent trend emerges regarding the evolution of genetic load: some invasive populations show patterns compatible with purging, while others appear to have accumulated deleterious alleles. These contrasting patterns highlight the complexity of evolutionary trajectories during invasions.

Conclusions: Our multi-species comparative approach suggests that the dynamics of genetic load during invasions are highly context-dependent. While purging may contribute to invasion success in some species, it does not appear to be a systematic outcome. Ongoing work will refine these findings and explore whether specific subsets of genes are more likely to undergo purging. These results emphasize the need for a more nuanced understanding of how demographic and selective forces interact during biological invasions.



Hybridization at the front wave of expansion increases genomic variability within an invasive species of slug, overcoming genetic drift

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POSTER SESSION 1 - P01.054, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

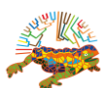
Background: Biological invasions pose significant challenges to biodiversity leading to the extinction of native species and to altering ecosystem function. When invasive species colonize new environments, they experience both strong founder effects and demographic expansions, contributing to genetic drift and thus hindering the efficiency of natural selection. Although invasive species are clearly successful in rapidly adapting to novel environments, the source of genetic variation facilitating invasiveness remains unclear. During invasion, hybridization between closely related native and invasive species often occurs, potentially counteracting genetic drift within invasive species and supplying locally adaptive variants that were already filtered by natural selection, ultimately fuelling the invasion process. This study addresses this hypothesis using the invasion of the invasive slug species *Arion vulgaris* that interacts with local subspecies of *A. ater*, leading them to extinction throughout Europe and highly impacting the local ecosystem because of its high predatory behavior.

Materials and Methods: We utilized a comprehensive sampling of the entire known species range sequenced for the whole genome at low coverage to assess delimitation of invasive species and native subspecies and identify possible hybrids between the taxa. Demographic analyses were used to determine the pattern of expansion for invasive species and the pattern of contraction for native subspecies. High coverage data for reference samples was used in determining the most probable species tree, and to quantify introgression between the invasive and different native subspecies using low coverage data. We estimated changes of genetic variability over time and space resulting from the combined action of genetic drift and introgression between native and invasive species, along the range of invasion.

Results: The genetic structure shows that hybridization is a widespread phenomenon in this

system, happening not only between the invasive and native species as a consequence of the invasion process, but also naturally between native subspecies. Demographic analyses showed that the invasive species is going through a process of demographic expansion while native species present a bottleneck, suggesting that their demographic dynamics are correlated. Aside from the ongoing hybridization, we also identified significant introgression between pure individuals of the invasive *A. vulgaris* and the native *A. a. ruber*. Interestingly, despite the expected drift caused by expansion, the genetic variability within the invasive species increases along the invasion wave, which supports the hypothesis that introgression from native into invasive species effectively prevents the loss of diversity.

Conclusions: Our results on the invasive slug *A. vulgaris* over the range of the native subspecies of *A. ater* offer general insights into the sources of genetic variability in the invasive species that potentially allow rapid adaptation during invasion. The invasion of the foreign species into a new habitat is accompanied by a demographic expansion and an initial loss of genetic diversity and increased genetic drift, as only a small fraction of the original population initiates the spread. However, our findings indicate that hybridization between congeneric native and invasive species during the invasion process may counteract this loss by rapidly increasing genetic diversity at the front wave of the invasion process and providing novel genetic combinations within the invasive taxon. Such introgression transfers locally adaptive alleles and can create a genetic substrate for rapid adaptation. This phenomenon, therefore, may be an important mechanism to explain the rapid adaptation of invasive species to novel environments, even under seemingly unfavorable demographic conditions.



Impact of dispersal and spatial processes on niche expansion during invasions

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POSTER SESSION 1 - **P01.061**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Invasive species can have widespread impacts on the ecosystems they enter. However, the process of invasion can also push invaders to evolve. Invasions are potent eco-evolutionary forces, and the process of expanding can lead to evolution even in the absence of the pressures of entering a new community. One empirically established example of this is increased dispersal speed in expanding populations, such as the invasive cane toads in Australia. In this study, we combine knowledge of dispersal evolution during invasions with emerging theory about evolution of niche expansion towards generalism during expansions. Using individual-based simulations, we allow dispersal distance and generalism to evolve individually and then together in heterogeneous landscapes. We expect dispersal distance to increase during invasions, and generalism to be more likely

to evolve. Individually, we find that both traits evolve during expansions as individuals take advantage of opportunity in the less crowded, more resource dense expansion front. When combined, there is the potential for these traits to be synergistic, with more dispersal leading to greater environmental heterogeneity and selection for generalism. However, in our model we see that these traits can also be antagonistic during invasions, with selection on reaching new areas first being so strong that it decreases co-occurrence of traits in the same individual. This study addresses how invaders can evolve during the process of invasions. Understanding the process of invader evolution can alter measures and understanding of invasibility, and create more complex null assumptions to compare against invader evolution within biological communities.



Introgression in the Anthropocene: detecting gene flow between native and alien flowering plants in the British flora

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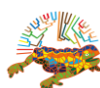
POSTER SESSION 1 - P01.052, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Human activities have altered species distributions drastically, and such changes often break down previous eco-geographical isolation between species, facilitating hybridisation between native and alien taxa newly brought into contact. Human-induced hybridisation can lead to contrasting evolutionary outcomes, ranging from the extinction of rare native taxa by demographic and genetic swamping, to the transfer of beneficial alleles that may assist local adaptation. Despite the fact that previous studies have evaluated introgression between native and alien taxa in a few illustrative cases, we still lack a holistic view on the prevalence and consequences of introgression across multiple taxonomic lineages. To address this knowledge gap, we present the first comparative study of human-induced introgression across multiple lineages of flowering plants at a floristic scale.

Based on floristic records, we target 88 known hybridising species-pairs that involve

a native and an alien plant taxon in the UK. Leveraging reference genomes from the Darwin Tree of Life project, we will generate whole-genome resequencing data to detect introgression using f-branch statistics and locate introgressed regions with window-based fdm scans. We will further test the adaptive value of introgressed regions by detecting signals of recent/ongoing selective sweeps. Finally, by integrating ecological and taxonomic trait data with the results of our genomic introgression estimates, we will develop a statistical model (MCMCglmm) to assess whether certain traits are associated with the extent of introgression.

This study will provide invaluable insight into the frequency, predictability, and evolutionary outcomes of gene flow between alien and native taxa at a floristic scale. The resulting statistical model will not only enable comparisons across diverse lineages for temperate floras, but may also help detect cryptic introgression.



Investigating morphological and behavioral spatial sorting in a predatory snake across a rapidly expanding invasion gradient

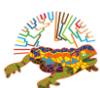
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POSTER SESSION 1 - P01.058, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Biological invasions offer exceptional opportunities to study rapid trait divergence. An intriguing process potentially underlying such divergence is spatial sorting, where individuals with higher dispersal tendencies tend to accumulate at the expanding front, leading to non-random trait distributions. Over time, this can result in actual shifts in allele frequencies across their invasive range. This rapid evolution could enhance spread speed with every generation. The recent invasion of the horseshoe whip snake (*Hemorrhois hippocrepis*) on the island of Ibiza (Spain) provides a compelling case to study this intriguing evolutionary process. Introduced around 2003, this top predator rapidly colonized much of the island, driving rapid local extirpation of the island's native fauna, including the endemic Ibiza wall lizard (*Podarcis pityusensis*). Here, we examine spatial sorting by examining whether the invading snake population is undergoing shifts in morphology and behavior between the front of invasion (the expanding edge) and the core of invasion (the oldest established area). Using an extensive field dataset—including an unprecedented sample size for a vertebrate predator—we

focus on ecologically relevant traits related to dispersal and foraging, including boldness, exploration, refuge use, and body shape. Despite their likely evolutionary relevance, behavioral traits remain largely understudied in invasion biology. By integrating them with morphological data, we gain a deeper understanding of the mechanisms underlying phenotypic divergence across the invasive range. We report differences in the morphology and behavior of snakes from the front of invasion compared to those from the core, which may be linked to variations in prey consumption and movement strategies. These patterns, observed along a clear core-to-front invasion gradient and over a remarkably short timescale, provide rare insight into how rapid phenotypic shifts emerge during the early stages of invasion. Our results contribute to shed light into our understanding of how invasive species can rapidly evolve after successfully becoming established in new environment, a crucial open question for global biodiversity conservation given that predator-driven invasions are the leading cause of island extinctions worldwide.



Maternal care thwarts parasitoids in the invasive brown widow spider (*Latrodectus geometricus*)

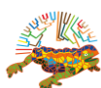
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POSTER SESSION 1 - P01.056, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Maternal care can maximize offspring survival and may contribute to the establishment success of invasive species. The brown widow spider, *Latrodectus geometricus*, is a successful invader worldwide. Here, we investigated the role of maternal care in enhancing its success. We compared the defence mechanisms of the invasive *L. geometricus* with those of another widow spider native to the Negev desert, *L. pallidus*, against an egg sac parasitoid wasp. Both spider species exhibited guarding behaviours following exposure to wasps; however, only *L. geometricus* efficiently evaded and successfully killed the parasitoid. Accordingly, its egg sacs were parasitized less frequently than those of *L.*

pallidus. Next, we evaluated the defensive role of the silk spike-like structures on *L. geometricus* egg sacs. When spikes were removed from half of the egg sac surface, the wasps laid more eggs on the spike-free side. In an additional experiment, *L. geometricus* females increased spike density on subsequent egg sacs after exposure to the parasitoid. We showed that *L. geometricus* employs both behavioural defences and modifications to the egg sac structure to protect against the parasitoid. These defences may provide *L. geometricus* an advantage over native species in its invasive range, potentially contributing to its invasion success.



Model-based demographic inference of recent invasions from genomic data

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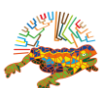
POSTER SESSION 1 - P01.065, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Invasive species are a major threat to biodiversity and a central topic in evolutionary biology. Biological invasions can be viewed as large-scale, unplanned experiments on how populations evolve to adapt to novel environments. However, in order to fully understand the evolutionary dynamics of invasions, it is essential to develop statistical tools that can disentangle the effect of selection and demographic processes in genetic variation.

In this work, we focus on the study of very recent invasions (that occurred less than 100 generations ago) using genomic data. More specifically, we aim to reconstruct the recent change in effective population size (N_e). Accurate estimates of N_e inform about the evolutionary history of a population and can guide conservation management plans, for example, by identifying the time of invasion and the rate of population growth. However, we found that the existing general-purpose methods that use linkage-disequilibrium (LD) and identity-by-descent (IBD) data have important limitations when

applied to realistic recent invasion scenarios.

We conducted extensive simulations to evaluate the limitations in the current methods and develop a novel approach specifically tailored to recent invasions that overcomes some of the observed issues. Our approach leverages coalescent theory to estimate key aspects of the invasion history from LD data and it is suitable for datasets comprising unphased genetic information from dozens to hundreds of individuals. Together with IBD data, we found that the estimated N_e trajectories can be used as a baseline to identify regions under recent positive selection. Moreover, we explored how approximate Bayesian computation methods based on IBD data can be used to study the dynamics of multiple spatial or temporal introductions. This work highlights the potential and limitations of demographic inference in the context of recent invasions and contributes to the development of tools for understanding rapid evolutionary processes in natural populations.



Phylogenomics and Trait Evolution in *Kalanchoe* (Crassulaceae): Insights from Whole-Genome Sequencing and Hybridization Analyses

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POSTER SESSION 1 - P01.066, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Kalanchoe (Crassulaceae) is a diverse genus comprising nearly 200 species, predominantly endemic to Madagascar, with others distributed across continental Africa and parts of Asia. Despite its ecological and economic significance—ranging from horticulture to model systems for Crassulacean Acid Metabolism (CAM) photosynthesis and invasion biology—trait evolution and a stable phylogenetic framework accounting for reticulate evolution for *Kalanchoe* remain unresolved. To address these gaps, we present the most comprehensive phylogenomic analysis of *Kalanchoe* to date. We leveraged de novo whole-genome sequencing for 59 taxa, supplemented by 48 newly generated internal transcribed spacer (ITS) sequences and publicly available data. This yielded nuclear low-copy genes, plastomes, and ITS datasets for 90, 94, and 251 taxa, respectively. Phylogenetic inference based on these datasets revealed topological incongruences and high discordance, prompting further investigations into polyploidy and hybridization. Using ploidy

estimation from gene recovery, as well as parsimony, maximum likelihood, and Bayesian approaches, we identified several ancient allopolyploidization events across five subgenera, some of which coincide with reduced reliance on sexual reproduction. Our trait evolution analyses further elucidate the origins and diversification of *Kalanchoe*'s asexual reproduction modes. Four specialized clonal propagation strategies—virtually unique to the genus—evolved independently in monophyletic Malagasy lineages, whereas others exhibit convergent evolution or ancestral retention. Reversals in specialization suggest trade-offs between clonal and sexual reproduction. Additionally, biogeographic reconstructions support Madagascar as the ancestral range of *Kalanchoe*, with a single dispersal event out of Madagascar and two independent expansions into Asia via North-East Africa. This study highlights the power of phylogenomics in resolving complex evolutionary histories and underscores the role of hybridization and polyploidy in shaping trait evolution in *Kalanchoe*.



Reconstructing the Origins of Contrasting Colonization Events in *Littorina saxatilis*

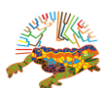
Sammarco B¹, Biello R¹, Carmelet Rescan D², Garcia Castillo D³, Westram A^{3,4}, Bosso L⁵, Butlin R^{6,7}, Johannesson K⁶, Mizzan L⁸, Macieira De Faria R⁹, Reeve J¹⁰, Benazzo A¹, Raffini F²

¹University Of Ferrara, ²Stazione Zoologica Anton Dohrn, ³Institute of Science and Technology Austria (ISTA), ⁴Nord University, ⁵CNR - ISAFOM, ⁶University of Gothenburg & Tjärnö Marine Station, ⁷University of Sheffield, ⁸Museum of Natural History of Venice, ⁹CIBIO/InBIO, Research Center on Biodiversity and Genetic Resources, University of Porto, ¹⁰Department of Marine Sciences at the University of Gothenburg

POSTER SESSION 1 - P01.057, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

In the current context of rapid environmental changes and increasing anthropogenic impact, understanding the evolutionary mechanisms underlying the uneven distribution of biodiversity is crucial to predict the redistribution of species, particularly why some populations are struggling to survive and/or adapt to changing environments, while others persist and even expand beyond their native range. To clarify how historical and genetic factors can drive colonization events, we analyzed available whole genome resequencing data from 475 *Littorina saxatilis* individuals collected from all over the North Atlantic region. This marine gastropod provides a powerful natural experiment of repeated colonization events across diverse environments, involving both recent and older range expansions, as well as some successful and some failing ones. We focused on the source of two range shifts: a failing recent introduction in the Venetian Lagoon (Mediterranean Sea, Italy), where the species represented the first confirmed alien species but is facing local extinction, and a successfully established colonization in Bodø, which has been settled there since the post-glacial range expansion in northern Norway. We investigated the origin of these two target populations, conducting analyses to reconstruct and clarify the colonization

routes and to compare genetic diversity between native and introduced populations. We observed that the south-eastern coast of England - particularly a lagoon area on the Norfolk coast - and possibly Mölle (Sweden) may have served as the source of the variability observed in the Venetian population, while Bodø most likely originated from a northward colonization by populations from the southern Norwegian coast or eastern UK populations. The 'stepping-stone' colonization pattern observed in Norway may have facilitated the successful establishment in Bodø, unlike the Venice population, which does not show evidence of intermediate stepping-stone populations - except potentially a lagoon population like Dersingham on the Norfolk coast. Interestingly, a long-distance dispersal hypothesis has also been considered for the Venetian population, proposing that its introduction might have occurred via maritime traffic or even through migratory birds acting as vectors. Our results enhance the evolutionary understanding of biological invasions and range shifts in *L. saxatilis*, help predict colonization outcomes, and allow us to draw conclusions about evolutionary dynamics that distinguish successful from failing range shifts, as well as local adaptation to new environments.



The Genomic Basis of Success and Failure in Marine Snail Range Expansions

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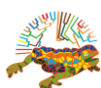
¹University of Ferrara, ²Stazione Zoologica Anton Dohrn, ³CNR - ISAFOM, ⁴University of Sheffield,

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POSTER SESSION 1 - P01.059, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Anthropogenic disturbances and climate change are dramatically altering species distributions, yet the mechanisms determining why some populations successfully expand their ranges while others fail remain unresolved. We address this fundamental question using the marine snail *Littorina saxatilis*, which exhibits repeated natural colonization events, both successful and failed, across diverse environments. Our main goal is to uncover the mechanisms underlying successful versus failed colonisations in this species, clarifying the evolutionary forces that drive range expansion or contraction. Our study integrates 380 newly sequenced samples from previously unstudied areas (including introduced populations), combined with 420 existing genomes to create a comprehensive dataset of 800 individuals. By combining whole-genome resequencing data (SNPs

and structural variants), phenotypic trait variation, demographic modelling and fine-scale environmental data, we aim to: (1) identify signatures of diversity loss, genetic load, and local adaptation; (2) assess how demographic history, genomic architecture, and recombination rates shape selection efficiency; and (3) determine whether evolutionary background, chromosomal rearrangements, and adaptive traits predict colonization success. Our findings will provide novel insights into the evolutionary mechanisms that facilitate or constrain range shifts in response to environmental change. Moreover, this work will advance our understanding of biodiversity dynamics and have critical implications for predicting species responses to ongoing global change, informing more effective wildlife management strategies.



Transcriptomic Evidence for Enhanced Gut Function in Non-Native House Sparrows

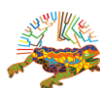
McCain K¹, Kohl K², Schrey A³, Martin L¹

¹University South Florida, ²University of Pittsburgh, ³Georgia Southern University

POSTER SESSION 1 - P01.064, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Range expansions and biological invasions serve as powerful natural experiments for examining how organisms respond to novel ecological pressures. The house sparrow (*Passer domesticus*), one of the world's most widespread and successful invaders, provides an ideal system to explore how changes in gene expression supports individual survival and persistence in newly colonized habitats. As organisms expand into new environments, they encounter novel food resources, but food consumption carries inherent risks, particularly the potential for exposure to parasites. To mitigate these risks, we hypothesized that invaders likely have specific physiological mechanisms that promote gut resilience in the face of pathogen exposure.

We conducted mRNA sequencing on duodenal tissue from 24 adult male sparrows across four native and four non-native populations. A differential gene expression analysis identified 558 genes with significant expression differences between population types, with 232 upregulated and 326 downregulated in non-native sparrows. Notably, the majority of the genes significantly up-regulated in non-natives are involved in processes supporting gut integrity, namely epithelial turnover, cytoskeletal modifications, and cellular anchoring. These functions are critical for maintaining barrier structure, enhancing nutrient absorption, and preventing microbial invasion. These results support the idea that non-native sparrows exhibit physiological shifts that promote gut resilience, helping them manage the risks associated with novel ecological conditions.



Transgenerational Effects of Sublethal Insecticides in the Colorado Potato Beetles

Margus A¹, Kankare M¹, Tikka S¹, Karvanen J¹, Lindström L¹

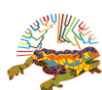
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POSTER SESSION 1 - P01.060, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The evolution of insecticide resistance in the pest species has historically been attributed to strong directional selection from lethal insecticide concentrations. However, emerging evidence suggests that sublethal doses may also drive resistance through the hormetic effects. Our previous research demonstrated that

transgenerational exposure to sublethal doses in the invasive Colorado potato beetle induces hormetic responses, including increased larva-to-adult survival, adult body mass, and egg hatching. We further investigated the pesticide effects at the transcriptome level to identify mechanisms behind these transgenerational hormetic changes. Sublethal insecticide doses affected only a small fraction of genes (approximately 0.1%), with minimal overlap between low- and high-dose groups. Low-

dose exposure influenced metabolism, reproduction, and detoxification pathways, while high-dose exposure induced mostly stress-related responses. Surprisingly, offspring from both parental exposure groups showed increased survival across generations. However, gene expression patterns diverged: the low-dose group displayed greater variation in the expression patterns, whereas high-dose group individuals were more consistent, suggesting stronger selective pressure. To conclude, individuals exposed to sublethal insecticide doses can produce offspring with increased insecticide tolerance, likely mediated through various pathways depending on the exposure level. These findings highlight the complex dynamics of insecticide resistance and the potential role of hormetic effects in pest management strategies.



Urbanization and the Evolution of Stoichiometric Flexibility in Trinidadian Guppies

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¹Department of Ecology and Evolutionary Biology

POSTER SESSION 1 - P01.067, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

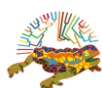
Urbanization alters nutrient cycling and energy flow in freshwater systems, creating novel environments that impose strong selective pressures on resident organisms. One of these pressures is eutrophication, an increase in nutrient load; yet, we know little about how urban organisms adapt to this increased nutrient content. We address this gap by investigating the evolution of stoichiometric flexibility, the ability to regulate body elemental composition in response to nutrient availability, in urban and non-urban populations of guppies (*Poecilia reticulata*). We hypothesize that urbanization selects for greater stoichiometric flexibility, allowing individuals from highly urbanized environments to capitalize on eutrophication by adjusting their body elemental composition to nutrient availability. We predict that urban fish will show stronger stoichiometric shifts under nutrient-rich conditions. In contrast, less urban fish will maintain more stable ratios and growth under nutrient-poor conditions, reflecting divergent plastic responses shaped by urban environmental pressures.

To test these predictions, we maintained, under controlled conditions, 144 F2 female guppies originating from wild populations across two rivers in Trinidad (Maracas and Tacarigua) and two urbanization origins (high- "HU" and low-urbanization "LU"). We

fed them with one of two artificial diets differing in nutrient content ("high-" and "low-quality") for 7 weeks. Females were then presented to males for five days and left alone for 4 weeks. We then quantified the nutrient allocation towards body and reproductive tissues (C:N, C:P, N:P), as well as fish growth rate before and after male introductions.

Fish from HU sites, especially those from the Tacarigua river, exhibited distinct elemental composition with lower C:N ratios, consistent with greater %N and potentially enhanced stoichiometric flexibility. In contrast, low-urbanization fish maintained more stable elemental profiles regardless of food quality, suggesting reduced responsiveness to nutrient enrichment. Although all fish showed increased %C and higher C:N when fed high-quality food, the response was not significantly different between origins, indicating similar flexibility in carbon allocation.

By integrating controlled experiments with stoichiometric, we aim to uncover the mechanistic basis of urban evolution in fish. Ultimately, this work will advance our understanding of evolutionary processes in urban ecosystems and highlight the importance of organism stoichiometry in shaping their success in rapidly changing environments.



A genomically informed prediction of the overwintering range of cotton bollworm in North America

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¹Department Of Zoology, University Of Cambridge, ²Selwyn College, University of Cambridge, ³Girton College, University of Cambridge, ⁴St John's College, University of Cambridge

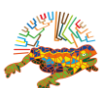
MEETING ROOM 120+121, August 18, 2025, 10:30 AM - 12:30 PM

Species distribution models (SDMs) are widely used in invasion biology to identify suitable habitat and predict where an invasive species might spread after introduction. A key limitation of existing SDM methods is their assumption of niche uniformity throughout a species' range, with no consideration of evolutionary adaptation to local environmental conditions. This can lead to uninformative or potentially misleading predictions. As high-throughput DNA sequencing has become cheaper and more accessible, interest has grown in developing better SDMs that incorporate information about local adaptation from population genomic data. We leverage whole-genome resequencing data from over 1500 individuals to create genomically informed predictions of the present and future overwintering range of an invasive crop pest in North America, accounting for local adaptation.

The cotton bollworm *Helicoverpa armigera* is one of the world's most economically damaging agricultural pests. In its native range, it is widely distributed across Africa, Eurasia, and Australia, but is also invasive in South America. Of particular concern is a recently established population at Chicago

O'Hare Airport, which has the potential to spread throughout North America. We demonstrate that the recently established North American population carries a structural variant on the Z chromosome, which is locally adapted to warm winter conditions in *H. armigera*'s native range. We use this information to create a genomically informed SDM to assess habitat suitability across North America in the present and under future climate change. We find that incorporating genomic information about local adaptation decreases predictions of habitat suitability for *H. armigera* in North America, but that short-term climate change increases its risk of spread from Chicago regardless of local adaptation.

This research has resulted in a more refined assessment of at-risk areas from this invasive crop pest, with implications for agricultural management practices both now and in the future. Additionally, our methodology represents a novel and robust way of creating evolutionarily informed SDMs by explicitly incorporating information about local adaptation from population genomic data.



Bidirectional exchange of resistance genes between native *Helicoverpa zea* and invasive *H. armigera*

North H¹, Amado D¹, Montejo-Kovacevich G^{1,3}, Jiggins C¹, Soares Correa A²

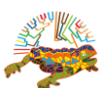
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MEETING ROOM 120+121, August 18, 2025, 10:30 AM - 12:30 PM

Invasive species pose significant threats to biodiversity and food security. Though regrettable, they also present remarkable evolutionary experiments. Population genomic data are increasingly being used to study biological invasion to quantify rapid adaptation in 'real time', while simultaneously informing efforts to monitor, manage and mitigate invasive pests. Noctuid moths of the genus *Helicoverpa* are among the most economically damaging crop pests globally. The invasive species *Helicoverpa armigera* was first officially reported in Brazil in 2012. There, it hybridized with its native sister species *H. zea*. We generated a 10-year time-series dataset spanning the course of the biological invasion. These data consist of over 1000 whole genome sequences from

Helicoverpa samples collected at 10 locations throughout Brazil. Although interspecific hybridization was rare due to pre- and post-zygotic reproductive barriers, occasional hybridization was sufficient to result in substantial inter-specific gene flow. Through adaptive introgression, synthetic pesticide resistance alleles were introduced to native *H. zea* from invasive *H. armigera*. More recently, alleles conferring resistance to Bt crops were introduced to invasive *H. armigera* from native *H. zea*. Despite the stability of species boundaries in sympatry, this exchange of fitness-enhancing alleles resulted in rapid adaptation over ecological time-scales, contributing to the spread of both pest species.



Invasion dynamics of the house mouse in Africa

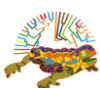
Poveda Martinez D^{1,2}, Rougeron V^{3,4}, Brouat C^{1,2}, Prugnole F^{3,4}

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MEETING ROOM 120+121, August 18, 2025, 10:30 AM - 12:30 PM

The house mouse (*Mus musculus domesticus*), a commensal rodent, has become one of the most widespread and successful invasive mammals worldwide. Its range expansion has closely followed human migration patterns, first during Antiquity and later during European colonial expansion. While genomic studies have uncovered significant insights into the colonization patterns of *M. m. domesticus* across Europe, North America, and oceanic islands, its invasion history in Africa has remained largely unexplored, despite Africa's central role in human trade and migration. In this study, we present a large-scale population genomic analysis based on 416 whole-genome sequences, including 333 new genomes from 13 African populations. Through genome-wide genetic population analyses and demographic reconstruction, we uncover evidence for at least three independent colonization events into Africa from distinct Eurasian sources. North African populations (Morocco, Algeria) and Niger show genetic ancestry tracing back to the Iberian Peninsula; Tunisian populations are linked to Eastern Mediterranean ones; and populations from West and Central Africa

(Senegal, Mali, Gabon, and Benin) cluster with Northern European lineages. Our analyses also reveal signals of introgression from the native *Mus spretus*, especially in regions of current sympatry such as Tunisia and Morocco. These introgression signals gradually decline from north to south within Africa. Demographic analyses suggest severe bottlenecks associated with each colonization wave. The timing of these events aligns with known historical human movements: ~800 years ago in North Africa, and ~200–600 years ago in West Africa, the latter being consistent with colonial-era introductions. Overall, our findings reveal a complex history of *M. m. domesticus* in Africa, shaped by multiple introduction events in different areas, hybridization with native species, and the signature of human-mediated dispersal. By incorporating a dense genomic sampling across Africa, our study fills a major gap in the global invasion history of *M. m. domesticus*. We provide the final missing piece of the puzzle, positioning Africa as a crucial region in understanding the evolutionary dynamics and human-mediated dispersal of this commensal species.



Rapid adaptation in an island endemic lizard in response to an invasive predator?

Talavera A¹, Casbas G¹, Vez M¹, Amor-Jiménez C¹, Giska I¹, Lapiedra O¹

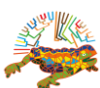
¹CREAF (Centre for Ecological Research and Forestry Applications)

MEETING ROOM 120+121, August 18, 2025, 10:30 AM - 12:30 PM

Evolution is most often envisaged as a slow-paced phenomenon, and its study is therefore usually restricted to long time-scale comparisons. However, research on evolutionary biology has witnessed a recent paradigm shift: the Anthropocene has shown us that evolution is not only a gradual, slow process, as Darwin had hypothesized. Instead, human-induced rapid environmental changes have helped us understand that evolution can in fact happen in contemporary timescales, with biological invasions being one of the major drivers of evolutionary shifts.

Whereas rapid adaptation of invasive species to new environments has received increased attention, the extent to which native species may adapt to their invasive predators remains largely unexplored. By

leveraging an ongoing invasion of horseshoe whip snakes (*Hemorrhois hippocrepis*) in the island of Ibiza (Spain), we assessed the evolutionary response of their most common native prey, the Ibiza wall lizard (*Podarcis pityusensis*), a well-known example of island tameness. Using X-rays and newly designed behavioral assays, coupled with Whole Genome Sequencing data from over 300 individuals of this endangered lizard, we examined: 1) morphological and behavioral shifts throughout the snake invasion, 2) candidate SNPs associated with those changes, and 3) the presence of detectable selection signatures in response to the invasion. Our study provides valuable insights into how island endemic species can adapt facing dramatic predation regime shifts.



Short and long term effects of an invasive parasite on the physiology of host fish populations.

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Homogenisation of communities, loss of genetic structure and decline or extinction of endemic species are some major threats presented through the spread of invasive species. Invasive parasites pose an acute challenge to native hosts. *Sinanodonta woodiana*, an invasive freshwater mussel from East Asia, has exhibited an explosive global spread including many European water bodies. *S. woodiana* has a generalist parasitic larval stage (glochidium), taking advantage of naïve European fish hosts including the European bitterling, *Rhodeus amarus*. *R. amarus* is a native European cyprinid fish that exhibits an intimate reciprocal relationship with unionid mussels. Bitterlings spend most of their lives in close proximity to mussels as a result of their unusual reproductive biology when they lay their eggs into mussels' gills. Bitterlings coevolve with native mussel partners and are able to minimize the infection of mussel glochidia. Glochidia decrease the physiological state of their fish hosts and the invasion by *S. woodiana* presents a new challenge to European bitterlings compared to native unionids. Our study included three populations of bitterling with varying levels of sympatry with *S. woodiana* to test the role of rapid coevolutionary response in terms of host defence and the impact of the invasive

parasite on physiological state of *R. amarus* individuals. Using experimental infections, we measured short-term (immediate survival and swimming capacity) and long-term (overwintering survival, growth rate, and condition) impacts on host physiology. Our study demonstrates a significant difference in short-term and long-term effects of *S. woodiana* glochidia on *R. amarus*. Compared to control fish, parasitised fish showed lower survival and swimming performance immediately after infection. The only population difference was observed in the number of glochidia attached on the fish, where the population with longest sympatry with *S. woodiana* had the least glochidia attached. In contrast to these findings, surviving *R. amarus* seemed to be generally unaffected by the parasitic infection later in their life, in terms of overwinter survival, physiological condition, reproductive allotment and growth rate. Overall, we did not observe a clear population specific role of rapid coevolution but a significant impact on short term survival and swimming performance. The lack of negative impacts in the long-term consequences of glochidia parasitism could be a result of higher mortality of infected fish in early stages, and hence eliminating weaker individuals (selective disappearance).



Unusual genetic structure of the alien plant *Cotula coronopifolia* in Europe, resulting from selfing, introduction history and dispersal by migratory waterbirds

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MEETING ROOM 120+121, August 18, 2025, 10:30 AM - 12:30 PM

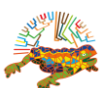
Migratory waterbirds play a significant role in the long-distance dispersal (LDD) of plants lacking fleshy fruits. However, their influence on the spread and genetic connectivity in invasive species remains poorly understood. We selected for our study the buttonweed *Cotula coronopifolia*, a species native to South Africa and introduced into Europe over two centuries ago. Although the species is predominantly coastal, it is expanding inland in Sweden and the UK. Faecal analysis supports dispersal by waterbirds as a key role for spread since the initial introduction to Europe in the 18th century. However, it is also commercially available as a pond plant.

We selected a total of 26 populations of *C. coronopifolia* across six European countries (Sweden, Germany, Netherlands, UK, Portugal and Spain) and four populations within its native range in South Africa. Genotyping by sequencing (GBS) was conducted on 266 individuals, generating extensive sequence and single nucleotide polymorphism (SNP) data. We estimated the genetic variability of populations and evaluated their genetic structure using the Bayesian clustering method implemented in the program STRUCTURE. We used STAIRWAY PLOT to infer the demographic trajectories of the different genetic clusters (=lineages) identified by STRUCTURE analyses and BPP to reconstruct their phylogenetic relationships and estimate divergence times.

Our results show the presence of three main lineages across the invaded range in Europe, whereas each population sampled within the native range in South Africa formed its own genetic cluster. Some individuals and populations show signals of admixture among different genetic clusters, which often translates into higher levels of genetic diversity. However, an initial increase of genetic diversity following admixture

seems to be erased after a few generations due to the predominantly selfing nature of the species. Phylogenetic analyses revealed that one population from South Africa diverged from a clade comprising all other lineages during the last glacial period (ca. 40 ka BP). This clade then split almost simultaneously into four lineages around the Last Glacial Maximum (ca. 23ka), followed by the divergence of two lineages currently restricted to the invaded range in Europe during the Middle Holocene (ca. 6 ka BP). Demographic reconstructions indicate that effective population sizes of European populations have remained constant since the documented arrival of the species to the continent (1739). Native populations exhibit idiosyncratic demographic trajectories and have experienced declines, expansions or bottlenecks since the onset of the Holocene.

Our analyses indicate a predominantly self-crossing nature of *C. coronopifolia*, which results in very limited admixture among genetic clusters and extremely low levels of genetic diversity. Estimation of divergence times suggests that multiple introductions may have occurred, likely facilitating the establishment and spread of the species in Europe. Sharing of clusters between coastal and inland sites connected by waterbirds as well as the presence of three genetic clusters in the Netherlands, which function as a nexus between other countries in migratory flyways, support the potential role of waterbirds in the spread of the species. Alternative hypotheses that were not supported include a higher connectivity between populations connected by ocean currents, which would be expected under sea dispersal, and higher diversity near major urban centres or ports, as would be expected under repeated contemporary introductions through shipping or horticulture.





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**S06 - Cancer in an evolutionary
framework: across species and within
individuals**



Evolution of Mutational Susceptibility to Genetic Diseases

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POSTER SESSION 3 - **P03.016**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Cancer is a genetic disease, remnant from a time before multicellularity, when individual cells prioritised their own growth over cooperative constraints. Major efforts in cancer genomics have focused on identifying cancer driver genes that can cause cancer when mutated. While extensive research has focused on how cancer is controlled once mutations arise in these genes, it remains unknown whether natural selection has also acted on the root cause: the probability of these mutations occurring in the first place. We are quantifying how mutation probabilities in cancer driver genes have evolved by reconstructing ancestral genomes across the primate and mammalian phylogenies. Focusing on key COSMIC mutational signatures SBS1 and SBS5, we find a decrease in susceptibility to

these processes from the human-gibbon common ancestor to modern humans. To distinguish cancer-specific selection from neutral evolutionary drift, we apply a fine-tuned model of neutral evolution. We further expand our analysis to investigate the broader question of mutational bias in germline processes for genes under varying germline selection pressures. This work introduces a new framework for understanding how mutational processes shape cancer risk and how germline evolution influences susceptibility to genetic diseases. It will further shed new light on Peto's paradox and may ultimately contribute to advancements in cancer prevention, early detection, and therapeutic strategies.



Intra-tumor evolution can favour inter-clonal cooperation and facilitate metastasis

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POSTER SESSION 3 - **P03.184**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Tumours are genetically and phenotypically diverse populations of clones that can also exhibit high levels of phenotypic plasticity in response to both microenvironmental cues and signals from each other or other non-cancer cells. This tumour complexity reflects in multifaceted evolutionary dynamics that ultimately result in increased individual fitness or collective success. Nevertheless, we are just starting to understand the implications of these phenomena. While inter-clonal competition may result in selective sweeps and a reduction in heterogeneity, recent findings indicate that cancer clones can also participate in cooperative interactions, which could contribute to maintaining tumor heterogeneity. Recently, we have addressed the role of inter-clonal cooperation in increasing the metastatic potential of tumours. Specifically, to investigate if and how genetically diverse clones cooperate during migration and invasion, we used distinct cancer cell lines expressing different metastatic capabilities. We found that the conditioned media from highly invasive cancer lines (breast and lung) enhanced the migration and invasion of less metastatic lines. This inter-clonal cooperation involved the transforming growth factor $\beta 1$ (TGF- $\beta 1$) signaling pathway. Moreover, co-culturing a less aggressive line with a highly metastatic breast line enhanced the invasive potential of both lines, facilitated by the co-option of the weakly metastatic clone into expressing an enhanced malignant phenotype that benefited both clones (i.e., a "help me help you" strategy). Based on our findings, we proposed a model in which crosstalk, co-option, and co-dependency can facilitate the evolution of synergistic cooperative interactions between genetically distant clones. Specifically, we suggest that synergistic cooperative interactions can easily emerge between genetically distant clones via crosstalk involving clones able to constitutively secrete molecules that induce and maintain their own malignant state (producer-responder clones) and clones that can respond to those signals (responder clones) and express a metastatic behaviour. Our findings also suggest that interfering with such cooperative interactions during the early steps in the metastatic cascade could provide additional strategies to increase patient survival. However, therapies targeting inter-clonal cooperation remain underexplored. Given that TGF- $\beta 1$ can transactivate the epidermal growth factor (EGF) signaling pathway, we investigated the potential of inhibiting the EGF receptors (EGFR) as a therapeutic intervention. We found that treatment with approved EGFR inhibitor drugs significantly reduced migration and invasion in both cell lines. To elucidate the molecular mechanisms underlying this cooperative interaction and identify additional genes that can be targeted therapeutically, we conducted transcriptomic analyses to (i) identify genes and pathways regulated during this inter-clonal interaction and (ii) assess the impact of EGFR inhibition. Overall, these studies underscore the importance of deciphering the nature and mechanistic basis of inter-clonal cooperative interactions that could take place during cancer progression. This is particularly relevant to metastasis, for which treatment options are limited, despite the fact that it is responsible for most cancer-related deaths.



Investigating the metastatic potential of two transmissible cancers in Tasmanian devils

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POSTER SESSION 3 - **P03.196**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

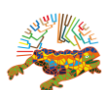
Background: The Tasmanian devil is one of the few species known to be affected by transmissible cancers, posing a critical threat to its survival. Two distinct cancers, Devil Facial Tumour Disease 1 (DFT1) and Devil Facial Tumour Disease 2 (DFT2), are transmitted through biting and have the unique ability to evade immune detection while spreading (metastasizing) within the host. Once established in a new host, these cancers can metastasize to internal organs, ultimately leading to organ failure and death. Despite their similar transmission routes, DFT1 and DFT2 differ in their rates of spread, proliferative and competitive capacity, within host tissue, and overall virulence. Remarkably, both cancers arose independently from Schwann cells, glial cells of the peripheral nervous system, approximately 30 years apart. This convergence in cellular origin within the same host species makes them an exceptional model for investigating tumour evolution, metastatic mechanisms, and tumour–microenvironment interactions. This study aims to: (1) Compare the metastatic potential of DFT1 and DFT2, (2) Assess their competitive interactions, (3) Investigate their communication with the tumour microenvironment, particularly macrophages and nerve cells.

Materials and Methods: An in vivo zebrafish embryo model was employed to study complex tumour–host interactions. Transgenic zebrafish lines with fluorescently labelled macrophages or neurons were transplanted with GFP-labelled DFT1 or DFT2 cells in the swim bladder. Proliferation, migration, and co-localisation were

monitored over four days using confocal and fluorescence microscopy. Images were analysed using ImageJ software.

Results: DFT2 cells showed high proliferation at the injection site within 24 hours and rapidly initiated migration into surrounding tissues. By day 2, cells were detected in multiple regions including the head, yolk sac, notochord, eye, kidney, and tail. These migrating cells often appeared in close proximity to both macrophages and neural tissue. In contrast, DFT1 displayed a delayed response, with limited proliferation during the first 48 hours and initial migration observed from day 3 onward. This suggests DFT2 may possess more intrinsic properties facilitating invasion and dissemination, or it may interact more effectively with the host microenvironment.

Conclusion: These early findings indicate that DFT2 exhibits a more aggressive metastatic phenotype compared to DFT1, aligning with previous in vitro studies. The use of a zebrafish model allows real-time visualization of tumour–host interactions, including proximity to macrophages and nerve structures, which may play a role in facilitating or constraining tumour spread. Ongoing analysis will explore how these interactions differ between DFT1 and DFT2, with a particular focus on neural tropism given both cancers' Schwann cell origins. This work may provide insights into the evolutionary pressures shaping tumour transmissibility and metastasis, with potential relevance to wildlife conservation but also human cancers involving nerve–tumour interactions.



No evidence that oestrogen positive breast cancer risk is genetically correlated with human life-history traits

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POSTER SESSION 3 - P03.202, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Oestrogen positive breast cancer (ER+) is one of the most prevalent cancers, but the evolutionary processes shaping genetic variation in ER+ risk are poorly understood. Here, we explore evidence for antagonistic pleiotropy between ER+ risk and human life-history traits, testing whether increased genetic ER+ risk is indicative of a reproduction-survival trade-off or fast life history. We estimate genome-wide genetic correlations between ER+ risk and three life-history traits (the numbers of children born, and age at menarche and first birth) using genomic restricted maximum likelihood analyses on Lifelines biobank women, and – for the first time – perform linkage disequilibrium score regression on European family-based genome-wide association study data. Regardless of the data or method used, genetic correlations were low and not statistically different from zero. Thus, we find

no evidence for antagonistic pleiotropy between ER+ risk and life-history traits, suggesting that these mutations have instead accumulated due to weak selection at older ages. We cannot rule out antagonistic pleiotropy shaping the evolution of individual ER+ risk variants but, given these traits' polygenic nature, our quantitative genetic analyses are best placed to assess its genome-wide importance, which appears minimal. Our results also suggest, at most, only the smallest changes in prevalence of genetic ER+ risk in future generations due to natural selection but future studies should examine how this genetic variation interacts with numerous environmental risk factors. In a landscape of ageing demographics and anthropogenic changes, continued study of ER+ risk factors is vital in the fight against cancer and the promotion of healthy ageing.



Organ-specific variability of tumor prevalence across wild mammals

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POSTER SESSION 3 - P03.191, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Cancer appears ubiquitous across the vertebrate's tree of life, yet susceptibility to this disease varies significantly among species, as for example mammalian orders exhibiting notably different cancer prevalences. Specifically, carnivorous species face higher cancer risk, potentially due to mutagens biomagnification, dietary composition, or pathogen-driven oncogenesis. In addition, many studies demonstrate a counterintuitive absence of associations between cancer risk and body mass or life expectancy, a phenomenon called Peto's paradox. However, it remains to be explored how cancer susceptibility in various organs scales with body mass or lifespan across species. Exploring this could also offer valuable insights into the evolution of cancer resistance mechanisms and clarify whether the previously demonstrated dietary influences on cancer risk are linked to organ-specific vulnerabilities or broader, systemic increases in cancer risk.

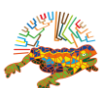
Materials and methods: To estimate tumor prevalence in different organs, we used medical data extracted from the Zoological Information Management System (Species360), providing a centralized international database of captive animals. We obtained 12,403 necropsy reports for 58 mammalian species, including 761 reports containing one or more neoplasia diagnosis, from which we extracted the organ affected by the tumors. Organs were gathered in 9 functional groups: lymphatic system, gastro-intestinal system, uro-genital system, skin, endocrine system, nervous system, respiratory system, circulatory system and fat tissues. Diet, body mass and life expectancy data were collected from the literature.

To assess tumor prevalence differences between phylogenetic orders, we estimated phylogenetic

signals for each group of organs, and performed pairwise order comparisons. When investigating diet effects, we used independent Phylogeny-corrected Generalized Linear Mixed Models (PGLMM) regressions. Each of the regressions were corrected by body mass and life expectancy. PGLMM regressions were also used to investigate Peto's paradox, using body mass or life expectancy as sole explanatory variables of tumor prevalence. All PGLMM regressions took into consideration the total number of necropsies available per species, as the tumor prevalence estimation is expected to be more precise with an increased number of necropsy records.

Results: We found a significant phylogenetic signal on tumor prevalence in the lymphatic system and the skin. Additionally, for the same two groups of organs, species in the Carnivora order show significantly higher tumor prevalences than those in Artiodactyla and Primates. When assessing the effect of diet on tumor prevalence for each group of organs, the consumption of Mammals as a primary or secondary food item was positively correlated with tumor prevalence in the lymphatic system, the GI system, the skin, and the endocrine system. Finally, for all groups of organs, no significant relationship was found between tumor prevalence and adult body mass or life expectancy.

Conclusions: Our results reveal that the increased risk of cancer observed for Carnivora in previous studies is more likely to be associated with a systemic increased risk of cancer, rather than only in the digestive system. Finally, as no relationship was found between tumor prevalence and body mass or life expectancy, Peto's paradox is validated at the scale of different organs, suggesting that the cancer resistance mechanisms evolved by bigger/longer-lived species are general to all organs across the body.



Pan-Mammalian genomic insights into cancer resistance and therapeutic target discovery

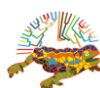
Padovani G¹, Smith L¹, Gilman N¹, Cahill J¹, Graim K¹

¹University of Florida

POSTER SESSION 3 - **P03.202**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Translating pre-clinical cancer discoveries into effective human treatments is particularly challenging, especially for rare cancers, which often lack enough patients to conduct clinical trials and build effective models. Comparative oncology leverages non-human animals to help model diseases such as cancer. However, identifying model organisms suitable for studying a particular disease can be difficult. For example, pre-clinical trials using the common mouse model have a 92% failure rate in Phase I trials. Utilizing mammals with higher clinical and genomic similarity to human diseases as non-traditional models, such as dog models of breast cancer and sarcomas, can yield more successful results and highlight the promise of comparative oncology. However, reliable identification of animal models most suitable for diseases such as cancer remains challenging. Suitable model organisms for a cancer will be those who share high genomic similarity with humans across genes associated with the given cancer. One way of identifying this genomic similarity is to analyze evolutionary information across

mammals. Here, we apply PhyloP across the Zoonomia 241 species alignment to identify conserved and accelerated regions across mammalian species and compare it to a null model with shuffled genomic information. Within each of the 19 Zoonomia orders, we do pairwise comparisons between species and perform enrichment analysis in human cancer genes by statistically evaluating PhyloP conservation and acceleration scores in all COSMIC cancer genes. We compare these scores to a randomly selected set of non-COSMIC cancer genes and repeat this comparison with replacement 100 times. This allows us to select the most enriched genes related to cancer in each species across the mammalian trees. From this, we identified pan-mammalian mechanisms of cancer resistance and susceptibility by analyzing the evolutionary history of cancer-related genes and pathways. Our results demonstrate that incorporating evolutionary information into model organism selection is a way to identify promising model organisms.



Preventing evolutionary rescue in cancer using two-strike therapy

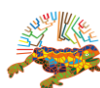
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POSTER SESSION 3 - P03.199, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

First-line cancer treatment frequently fails due to initially rare therapeutic resistance. An important clinical question is then how to schedule subsequent treatments to maximize the probability of tumour eradication. I will present a theoretical solution to this problem obtained by using mathematical analysis and extensive stochastic simulations within the framework of evolutionary rescue theory. Whereas standard clinical practice is to wait for evidence of relapse, we confirm a recent hypothesis that the optimal time to switch to a second treatment is when the tumour is close to its minimum size before relapse, when it is likely undetectable. This optimum can lie slightly before or slightly after the

nadir, depending on tumour parameters. Given that this exact time point may be difficult to determine in practice, we have studied windows of high extinction probability that lie around the optimal switching point, showing that switching after the relapse has begun is typically better than switching too early. I will further reveal how treatment efficacy and tumour demographic and evolutionary parameters influence the predicted clinical outcome, and insights into how best to schedule drugs of unequal efficacy. Our work establishes a foundation for further experimental and clinical investigation of this evolutionarily-informed multi-strike treatment strategy.



The cross-talk between ER+ breast cancer cells during progression

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*POSTER SESSION 3 - **P03.182**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM*

Metastasis to this day remains the primary cause of most breast cancer (BCa) related deaths. Estrogen receptor-positive (ER+) BCa shows a higher recurrence rate after five years of diagnosis than in the first five years. Regardless of the substantial research efforts, the exact mechanisms that allow cells to metastasise are still unclear. Recent studies indicate that metastases can arise from polyclonal seeding of multiple genetically distinct clones interacting with each other while they progress. Interestingly, both negative and positive interactions between those cells coincide, pointing out that cancer behaves according to concepts of evolutionary Game Theory, and should be

viewed as a living ecosystem. We aim to identify how stochastic the composition of those polyclonal clusters is in the context of ER+ BCa cells metastasising to the secondary organs, thus elucidating the cross-talk between those clones.

To study those processes, we use a variety of state-of-the-art techniques. We aim to trace each clone in vivo using multiple multicolour lineage tracing approaches and analyse the data with advanced microscopy, flow cytometry, single-cell RNA-seq and computational interaction analysis. Once cross-talk between the cells is identified, we will next focus on interrupting it to disrupt the metastatic progression process.



The role of the microbiome in transmissible cancers, a case study on Tasmanian devil facial tumour diseases

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POSTER SESSION 3 - **P03.187**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Tasmanian devil's (*Sarcophilus harrisii*) populations decreased by 80% over the last 40 years due to the emergence of two transmissible cancers, the Devil Facial Tumour 1 and 2 (DFT1 and DFT2). Currently, the Devil Facial Tumour Disease (DFTD), is present across more than 90% of Tasmania's territory. While extensive research has focused on the tumours' origin, evolution and disease epidemiology, since the discovery of the first cancer in 1996, the presence and impact of tumour and host microbiomes remain largely unexplored. In human cancer, microbes are known to influence cancer progression and therapeutic outcomes by affecting host immune responses to tumours. They have also been used as diagnostic markers for cancer development. We therefore propose that, similar to human cancers, the microbial communities may have a similar role and impact in DFTD. The aim of this project is to investigate interactions between the host, microbiome and tumour progression. First, opportunistically collected biopsy samples were used to characterise the microbiome of DFT1 and DFT2 tumours. By using a 16S amplicon sequencing approach the microbiome of a total of 50 DFT1/DFT2 tumour samples were analysed. Four times as many Amplicon Sequence Variants (ASVs), indicating a more diverse

microbiome community, was found in DFT2 than in DFT1 tumours. Investigating multiple tumours from the same devils also showed that the microbiomes of DFT1 tumours clustered together while the microbiome of DFT2 tumours were much more diverse. Next, the study will be focusing on identifying changes in asymptomatic and symptomatic Tasmanian devil gut microbiome over time to identify potential cancer biomarkers using faecal matter archived since 2012. Over 300 faeces samples, including more than 80 diseased devils, and some with several recaptures (including times where the individual was first confirmed disease-free and then later showing signs of tumour development) will be analysed. Full-length sequencing of the 16s and Internal Transcribed Spacer (ITS) genes, targeting the bacterial and fungal communities, are currently under way. The expected outcomes of this part of the project are the characterisation of the gut microbiome of asymptomatic and symptomatic devils along with the identification of potential markers of the cancer. The longitudinal samples will also allow for detecting changes in the microbiome along disease progression. Data analyses will be completed by August, and the results will be presented at the ESEB 2025 conference.



Using evolutionary accumulation models to identify therapeutic targets in cancer

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POSTER SESSION 3 - P03.024, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Cancer progression is caused by the sequential accumulation of somatic mutations during the life of an individual: cancer cells can accumulate mutations that increase cell-level fitness, increasing in frequency and spreading (and ultimately resulting in metastasis), at the expense of the whole organism.

Because of epistatic interactions, the accumulation of cancer mutations is often constrained to follow only some possible sequential orders, as the effect or viability of a mutation can depend on the presence of other mutations. Evolutionary accumulation models (EvAMs), also known as cancer progression models, have been developed to infer these dependencies in the order of accumulation of mutations during tumor progression. EvAMs use cross-sectional data and have been used in computational oncology, virology, and malaria progression. They have been applied to identify interactions between events, evolutionary routes of feature acquisition, and to improve patient stratification and survival prediction.

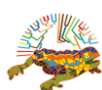
It has been suggested that EvAMs could be used to determine which genes to target by leveraging EvAMs' inferences about the evolutionary process. There is, however, no procedure in the literature for how to use EvAMs to this end, nor information on how EvAMs would perform.

Objectives, Approach, and Result: Here we explore the use of EvAMs to identify therapeutic targets. The focus is not on the quality of the inferences about the patterns of dependencies; instead, we want to know what could work from the point of view of interventions. We shift the focus from "correct" to "actionable" inferences. We define targeting a gene as making all genotypes that bear a mutation in that gene

lethal. This allows us to define a ground truth: for any fitness landscape, we can systematically examine the effects that targeting every gene has in terms of population composition. Then, given a specific objective (e.g., decrease the frequency of a particular genotype) we can rank how much targeting each gene contributes to this objective. Once we have the ground truth, we can assess EvAMs. An EvAM will be useful to identify targets if it can provide a ranking of gene interventions that is very similar to the true ranking of genes defined from the ground truth.

The protocol we use to identify the ground truth is an application of Pearl's do-operator, and our definition of effect is a simple rewording of Hernan and Robin's "causal effects in populations, that is, numerical quantities that measure changes in the distribution of an outcome under different interventions." To obtain the predictions under intervention from EvAMs, we show how to alter the fitted models so that they provide the counterfactual predictions under intervention; we discuss how to use a do-operator procedure under sensible modularity assumptions. We also address the difference between our definition of intervention ("making all genotypes that bear a mutation in that gene as lethal") and another intuitive definition of intervention as suppressing (or inactivating) the effects of a mutation. We show the performance of several state-of-the-art EvAMs under a simple strong selection, weak mutation model of evolution.

Conclusions: Our work highlights the opportunity to use EvAMs to identify therapeutic targets and the need to devise protocols to obtain explicitly causal predictions from these models, so they can be used in more complex evolutionary scenarios.



When rest fails, tumors rise: sleep-like state deprivation reveals an ancestral cancer-protective function in Hydra

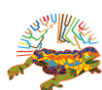
Meliani J¹, Berriahi Z¹, Renard J¹, Asselin K^{1,3}, Tissot S¹, Thomas F¹, Dujon A^{1,2}

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POSTER SESSION 3 - **P03.204**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Sleep is a behavioral and physiological state shared across the tree of life, but its evolutionary origins and core functions remain unclear. While sleep has been primarily studied in organisms with centralized nervous systems, sleep-like states have also been identified in early-diverging animals such as Hydra, a freshwater cnidarian with a simple body plan and no centralized brain. Hydra exhibits periods of behavioral quiescence structured not by circadian cycles but by endogenous ultradian rhythms—internal activity cycles shorter than 24 hours. This positions Hydra as a unique model to investigate the ancestral, possibly non-neural, functions of sleep-like states in multicellular life. Using mechanical stimulation, we chronically disrupted the sleep-like state in several Hydra species and monitored its impact on tumor development, phenotypic traits, and life-history parameters. Our main model, Hydra oligactis, included genetically distinct strains with varying spontaneous tumor susceptibility, including a lineage carrying vertically transmitted tumors. To adopt an evolutionary perspective, we also included three other species—H. viridissima, H. circumcincta, and H. vulgaris—that are not known to develop tumors, allowing comparisons across phylogenetically diverse, tumor-resistant backgrounds. Our results show that sleep-like state deprivation induces a marked increase in tumor incidence in H. oligactis, with tumors progressively growing over time. No comparable tumor-related effects were observed in the other species. Importantly, we did not detect significant changes in

budding rate or tentacle number, suggesting that sleep-like state deprivation does not overtly disrupt these specific life history traits under the tested conditions. This indicates that the effects of sleep disruption may selectively target tumor-related cellular processes without broadly altering morphology or growth patterns. These findings suggest that the sleep-like state in Hydra contributes to the regulation of cell proliferation and the suppression of tumor formation, even in the absence of visible effects on classical life-history traits. The contrast between tumor-prone and non-tumor-prone species supports the hypothesis that one of the ancestral evolutionary roles of sleep-like behavior could have been to protect multicellular organisms from cellular dysregulation — particularly neoplasia — possibly through interactions with other co-evolved physiological processes. From an evolutionary perspective, our results suggest that the link between behavioral quiescence and cancer resistance predates the emergence of centralized nervous systems and may represent a deeply conserved function of sleep-like states. In an organism like Hydra, where regeneration and cell turnover are tightly regulated, the loss of behavioral rest appears to disrupt key mechanisms of somatic maintenance. By uncovering this link in a simple yet highly regenerative model, our study offers new insight into the evolutionary pressures that may have shaped the emergence of sleep — not merely as a neural necessity, but as a fundamental mechanism for preserving somatic integrity in early multicellular life.



An unexplored connection between mutation, phenotypic plasticity and evolutionary convergence in cancer

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MEETING ROOM 122+123, August 22, 2025, 2:00 PM - 3:00 PM

All cancers are characterized by a series of shared phenotypic traits referred to as hallmarks of cancer. Because cancers that emerge and evolve independently within distinct individuals do not share a common ancestor (except for the few cases of transmissible cancers), the independent and recurrent expression of similar phenotypes (in the absence of similar patterns of genetic change or gene expression) suggests a high degree of convergence. Recently, unlocking phenotypic plasticity and nonmutation epigenetic reprogramming have been included in the list of emerging hallmarks and enabling characteristics of cancer. Generally, phenotypic plasticity can provide organisms a suite of adaptive traits in response to environmental changes. In addition, in multicellular organisms, individual cells can also express distinct stable or plastic phenotypes in response to developmental and microenvironmental cues. All cancers can independently access some of these normal cell phenotypes, and their expression in atypical contexts provides cancer cells with traits that increase their individual or collective success. Such phenotypes can be either stable (and heritable) or transitory (in response to specific signalling molecules from or microenvironmental stimuli). Nevertheless, the mechanisms underlying the plastic induction or stable expression of this unlocked plasticity are not well understood.

Building on our work on the evolution of multicellularity, I propose two new potential mechanisms that could contribute to the expression of normal plastic or stable phenotypes in cancer cells. Specifically, using data from experimentally evolved cancer cell lines, I will argue that normal pathways can be co-opted/expressed in atypical cellular contexts without the need for mutations or epigenetic modifications in genes associated with the typical expression of the phenotype itself. The co-option events can involve (i) microenvironmental stresses (for the plastic phenotypes) or (ii) independent nonspecific mutations (for stable phenotypes) that can elicit imbalances in the cell resulting in the activation of normal pathways through the fortuitous induction of the developmentally induced intracellular signal in a new/atypical context. Overall, the proposed scenarios bypass the need for specific parallel or convergent mutations for the evolution or expression of similar phenotypes. Such scenarios can switch the focus from searching for common genetic or epigenetic changes towards identifying the shared intracellular signals (e.g., changes in pH, redox status, levels of secondary messengers) associated with the independent expression of the same phenotype in both typical and atypical contexts.



Body size and sex differences affect cancer across species in lemurs

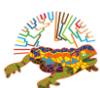
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MEETING ROOM 122+123, August 22, 2025, 2:00 PM - 3:00 PM

Oncogenic mutations during cell divisions make organisms susceptible to cancer. Yet, although larger size typically requires more cell divisions, larger species are not more cancer-prone than smaller ones. Selection to suppress cancer until ages with low reproductive value can explain this pattern and predicts stronger cancer defences in larger species. Here, we test this by taking advantage of a high-resolution cancer dataset. We conceptualise cancer defences as the number and rate of mutations that result in cancer, which we estimate by fitting a multistage cancer model to age-specific cancer data with a maximum likelihood

approach. We build 10 models that differ in how defences relate to body size, species differences and sex differences, and select the best-performing ones with an information-theoretic approach. Our results suggest stronger cancer suppression in larger species, allowing them to evolve longer cancer-free lifespans. Moreover, we find a higher rate of oncogenesis in female lemurs than in males. Our study supports the hypothesis that selection matches cancer defences to species life history, explaining the lack of correlation between species size and cancer incidence.



Estimating excess cancer risk: an evolutionary approach

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MEETING ROOM 122+123, August 22, 2025, 2:00 PM - 3:00 PM

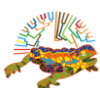
Background: For modern humans, the lifetime risk of many cancers has predictably increased with the rise in average lifespan; however, it has been noted that the prevalence of some cancers has risen disproportionately due to an additional increase in early-life incidence. It is important to know which cancers show this pattern so that we can begin to identify the causal factors. These cancers can be detected if their early-life increase has occurred over the modern period when recent epidemiological data can be compared to older data. A more general approach is to compare the current incidence of a cancer with the incidence expected in prehistoric times – which, of course, we do not know. However, we can use an evolutionary approach to estimate the maximum fitness loss expected before selection drives increased suppression of a given cancer. If the fitness loss that the current incidence pattern would have caused in a prehistoric human population is greater than the threshold value, then it is likely that some feature of modern human life has increased the incidence of that cancer.

Materials and methods: Prehistoric demography was estimated assuming that patterns of survival and fecundity were

similar to those estimated for hunter gather tribes, and varied according to the degree of parental care needed for offspring survival. These demographics were combined with worldwide incidence data for 70 different cancers in females and 66 in males to identify cancers whose modern incidence would have caused an excessive fitness loss in prehistoric times.

Results: It was found that approximately 10% of cancers caused an historical fitness loss above the estimated selection threshold. As expected, breast cancer in women was the disease causing the greatest potential fitness loss in a prehistoric population, while melanoma showed substantial geographical variation, with the highest fitness loss in Oceania (dominated by data from Australia); however, other results were less expected, such as the high potential fitness loss due to testicular cancer.

Conclusions: Those cancers with a high level of potential fitness loss are likely to have increased in frequency due to environmental or behavioural factors that have a greater influence on modern humans than on their ancestors. In some cases, the probable causes of the increase have been identified, but for others these urgently need to be identified.



Peristromal niches provide ecological rescue and facilitate the evolution of resistance to targeted therapies in lung cancer

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¹Moffitt Cancer Center

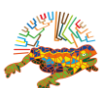
MEETING ROOM 122+123, August 22, 2025, 10:30 AM - 12:30 PM

Cancer therapies that target oncogenic signaling, such as inhibitors of ALK kinase (ALKi) in ALK mutant lung cancers, can induce strong and durable clinical responses. However, targeted therapies are not curative in advanced cancers as a subset of tumor cells typically survives, persisting within minimal residual disease, and eventually evolves resistance. While therapy resistance is typically attributed to cell-intrinsic (epi)mutational characteristics of tumor cells, it can also result from interactions of tumor cells with the tumor microenvironment (TME). In contrast to the detailed elucidation of the molecular mediators of therapy resistance, our knowledge of the evolutionary dynamics underlying the emergence of resistance remains poorly defined.

To dissect the relative contribution of cell-intrinsic and microenvironmental mediators of therapeutic resistance in vivo, we used mouse xenograft models of targetable lung cancers and histological analyses using tools adopted from spatial ecology. We found that therapy induced a profound shift in tumor cell proliferation towards peristromal niches, indicating an ecological rescue mechanism. Whereas this shift was most pronounced during remission, it was also partially retained during relapse, suggesting the direct contribution of peristromal niche to bona fide resistance (transition to net positive tumor growth). Mechanistically, partial protection of tumor cells within peristromal niches was

mediated by a combined effect of multiple juxtacrine and paracrine mechanisms that reduced drug sensitivity by activation of bypass signaling while also enhancing plasticity-mediated adaptation.

This protective effect of the peristromal niches was also a major modulator of the evolutionary dynamics of acquired resistance. Our in vivo cell competition experiments with spiked in resistant subpopulations revealed that the peristromal niche mediated an emergent positive ecological interaction between therapy-resistant and therapy-sensitive cells. By limiting tumor regression, the therapy-induced expansion of resistant cells limited the loss of protective peristromal niches, while the subsequent resumption of tumor growth created new peristromal niches capable of supporting the survival and proliferation of therapy-sensitive cells. The enhanced survival of therapy-sensitive cells potentiated their ability to adapt to therapies, potentiating the diversity of evolutionary trajectories towards resistance. Our results indicate that the acquisition of therapy resistance represents an eco-evolutionary process, thus requiring consideration of mechanistically complex cell-intrinsic and environmental effects. This complexity presents an inherent challenge to the success of current reductionistic approaches to cancer therapy, calling for the development of adaptive, evolutionary-informed therapeutic strategies.



Selection for function in cancer: a novel evolutionary principle with potentially groundbreaking therapeutic implications

Thomas F¹

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MEETING ROOM 122+123, August 22, 2025, 10:30 AM - 12:30 PM

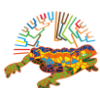
Cancer is classically viewed as a disease driven by Darwinian selection acting at the cellular level, where fitter clones outcompete others within the tumor ecosystem. However, recent work suggests that this vision is incomplete. Tumor progression is not solely shaped by the survival of individual cells, but also by their capacity to organize into functional groups — a concept captured by the notion of Group Phenotypic Composition (GPC).

The ability of a tumor to develop, persist, and resist therapies critically depends on this GPC — i.e., the spatial and functional organization of its internal heterogeneity. Selection therefore acts not only on individual cell traits but also on emergent properties at the collective level:

cooperation, division of labor, resistance networks.

This new evolutionary framework — selection for function — opens original therapeutic avenues. Rather than only trying to kill cancer cells, future strategies could aim to destabilize the tumor's GPC, pushing the system beyond a tipping point, leading either to its collapse or to a trajectory shift towards a less aggressive state.

Such a perspective invites system-level therapies targeting not just cells but their interactions, key functional nodes, or microenvironmental dependencies. It also offers promising opportunities for adaptive therapies, capable of steering tumor evolution, controlling resistance, and potentially transforming cancer into a manageable chronic disease.



Selective sweep probabilities in tumours and other range expansions

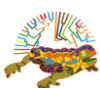
Stein A², Kizhuttill R^{3,4}, Bak M¹, Bostock K¹, **Noble R¹**

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MEETING ROOM 122+123, August 22, 2025, 10:30 AM - 12:30 PM

Evolution during range expansions shapes biological systems from microbial communities and tumours up to invasive species. A fundamental question is whether, when a beneficial mutation arises during a range expansion, it will evade clonal interference and sweep through the population to fixation. However, most theoretical investigations of range expansions have been confined to regimes in which selective sweeps are effectively impossible, while studies of selective sweeps have either assumed constant population size or ignored spatial structure. I will present results of using mathematical modelling and analysis to investigate selective sweep probabilities in various biologically relevant scenarios, including the unexplored case in which mutants can outcompete and displace a slowly spreading wildtype. Assuming constant radial expansion speed, we derive probability

distributions for the arrival time and location of the first surviving mutant and hence find surprisingly simple approximate and exact expressions for selective sweep probabilities in one, two and three dimensions, which are independent of mutation rate. Using agent-based simulations, we show that our analytical results accurately predict selective sweep frequencies in the two-dimensional spatial Moran process, and that they remain informative even when mutation effects on fitness are random and multiplicative. We further compare and synthesize our results with those obtained for alternative growth laws. Parameterizing our model for human tumours, we find that selective sweeps are predicted to be rare except during very early solid tumour growth, thus providing a general, pan-cancer explanation for findings from recent sequencing studies.



Sublineage dynamics and mitochondrial DNA exchange in Tasmanian devils' transmissible cancers

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MEETING ROOM 122+123, August 22, 2025, 10:30 AM - 12:30 PM

Background: Transmissible cancers are a rare type of cancers in which the living tumour cells are physically transmitted between genetically unrelated individuals. In Tasmanian Devils, carnivorous marsupials endemic to the island of Tasmania, two transmissible cancers, named Devil Facial Tumour (DFT) 1 and 2, are found and have led to endangerment of the species. These cancer lineages emerged several decades ago, and have been clonally spreading since then, and they thus provide a good opportunity to study the long-term evolution of cancer cells, and their diversification into different lineages.

Materials and Methods:

Based on the most complete sample set ever considered to study these transmissible cancers, with data gathered from over 2500 tumours and their matched hosts collected in the field for the past 20 years, we investigated the genetic diversity of DFT1 and DFT2 in the wild. Using a cost-effective method for genotyping tumours based on low-coverage whole genome sequencing data, we analysed the spatial and temporal dynamics of subclones. Besides, we assessed the mitochondrial DNA (mtDNA) diversity and reconstructed maximum-likelihood phylogenies based on mtDNA variants.

Results: First, we confirmed the geographic structure of DFT1 and DFT2 at the clade level and documented the changes in the frequency and spatial repartition of clades across Tasmania over time. Looking at a finer scale approach, we revealed distinct

patterns of sublineage replacement in different populations of Tasmanian devils. These patterns seem to be linked with environmental factors and movements of spreader devils across Tasmania. We also revealed the potential for coinfection and highlight the strong force of infection of Devil Facial Tumour Disease. This is valuable data to understand and predict the spread and evolution of DFT1 and DFT2 in the field and inform conservation strategies for the species.

Additionally, phylogenies based on mtDNA were found to approximately recapitulate the genetic relationships expected based on nuclear DNA, except for discrepancies which revealed the occurrence of mtDNA horizontal transfers from transient hosts into the DFT1 tumour lineage. We investigated the genetic and geographic origins of the mtDNA haplotypes thereby incorporated into the tumours. We considered the evolutionary consequences that these exchanges could have for this transmissible cancer, and suspect that this type of horizontal transfers could be more frequent than we think, even in non-transmissible cancers.

Conclusion: Using methods from molecular evolution and phylogenetics, this work sheds light on some of the factors underlying the transmission and genome evolution of cancers turned into an infectious disease. It illustrates how transmissible cancers are a privileged model to study cancer in an evolutionary framework, as well as contributing to a better understanding of the Tasmanian Devils' disease and the management options.



The permissive binding theory of cancer: an evolutionary theory of metastasis

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MEETING ROOM 122+123, August 22, 2025, 2:00 PM - 3:00 PM

The later stages of cancer, including the invasion and colonization of new tissues, remain especially mysterious compared to earlier stages like primary tumor formation. While we lack many details about both, we do have an apparently successful explanatory framework for the earlier stages: one in which genetic mutations hold ultimate causal and explanatory power. By contrast, on both empirical and conceptual grounds, it is not currently clear that mutations alone can explain the later stages of cancer. Can a different type of molecular change do better? Here, I will discuss the “permissive binding theory” of cancer, which proposes that novel protein binding interactions are the key causal and explanatory entity in invasion and metastasis. It posits that binding is more abundant at baseline than we observe because it is restricted in normal physiology; that any large perturbation to physiological state revives this baseline abundance, unleashing many new binding interactions; and that a subset of these cause the cellular functions at the heart of oncogenesis,

especially invasion and metastasis. Significant physiological perturbations occur in cancer cells in very early stages, and generally become more extreme with progression, providing interactions that continually fuel invasion and metastasis. The theory is compatible with, but not limited to, causal roles for the diverse molecular changes observed in cancer (e.g. gene expression or epigenetic changes), as these generally act causally upstream of proteins, and so may exert their effects by changing the protein binding interactions that occur in the cell. This admits the possibility that molecular changes that appear quite different may actually converge in creating the same few protein complexes, simplifying our picture of invasion and metastasis. If correct, the theory offers a concrete therapeutic strategy: targeting the key novel complexes. The theory is straightforwardly testable by large-scale identification of protein interactions in different cancers.





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S07 - Contribution of the microbiome to host adaptation and plasticity



A New Strepsipteran Parasite *Xenos gadagkari* sp.nov and its effect on Microbial Communities of Primitively Eusocial wasp *Polistes wattii*

Nain D¹

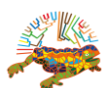
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POSTER SESSION 1 - P01.114, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Strepsipteran insects are obligate endoparasites that infect a broad range of insect hosts, often inducing striking morphological, physiological, and behavioural modifications. Within this order, the genus *Xenos* has been notably associated with eusocial wasps. While the parasitic interaction between *Xenos vesparum* and *Polistes dominula* is well documented, such associations have remained unexplored in the Indian subcontinent until now. Our research documents the discovery of a novel strepsipteran species, *Xenos gadagkari*, parasitizing *Polistes wattii*, a widespread and ecologically significant wasp in North India. We provide a detailed morphometric description and establish the phylogenetic position of *X. gadagkari* within Strepsiptera. In addition to characterizing this new host-parasite relationship, we explored their

microbiological dimension. We employed 16S rRNA gene amplicon sequencing using Oxford Nanopore Technologies to compare the microbial communities of healthy and *Xenos*-parasitized male and female *P. wattii*. Our findings uncover the significantly higher bacterial diversity in parasitized individuals, confirming altered microbial colonization due to parasitism.

This study represents the first report of *Xenos* parasitism in Indian *Polistes* and the first microbiome analysis of this genus. Our findings underscore the intricate ecological and evolutionary implications of host-parasite-microbiome interactions and highlight the potential of *P. wattii* and *X. gadagkari* as a novel model system for studying coevolution and symbiotic dynamics in social insects.



Alternative photobiont - where did it come from? Metabarcoding exploration of algal diversity living on lichens in the phyllosphere framework.

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POSTER SESSION 1 - **P01.094**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Lichens are iconic examples of symbiosis. Each thallus mainly comprises a fungus (heterotroph; mycobiont) and an algal and/or cyanobacterial partner (photoautotroph; photobiont). Lichens deposit extracellular polymeric substances (EPS) on the surface of their hyphae throughout the entire thallus, forming extracellular interaction matrices (EIM) that form the habitat for communities of algae, fungi, and bacteria. Furthermore, they form external and internal polysaccharide-dense zones depending on the structure of the lichen cortex. This feature allows the formation of intrathallic communities of algae, fungi, and bacteria (inside lichen thalli, under the cortex); and the formation of exolichenic biofilm (on the external part of the cortex) that includes the confirmed presence of bacteria and yeast, and probable presence of algal, and other filamentous fungal communities that in this area were not studied. Their existence was generally acknowledged but not verified; they were only considered contamination and removed.

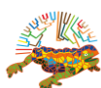
However, we lack information on the extent to which the exolichenic biofilm constitutes a part of the lichen holobiome, and how it is structured in the framework of the surrounding environment – the phyllosphere and rhizosphere. From the studies on lichen photobionts, we know that under stress conditions, lichens exchange the intrathallic algal partner for a better-adapted one acquired from the surrounding different lichen species. However, we lack knowledge of the purpose of exolichenic biofilm, which may be a source of adapted algae. Therefore, we aim to evaluate community variation and interaction of the lichen algal components, including their position inside or on the surface of lichen thalli under varying air pollution levels.

Materials and methods: Fifteen young *Acer platanoides*, with three *Xanthotia parietina* thalli on the bark (45 specimens in total), were selected in five locations. The material was collected between

6 and 8 August. A procedure of sampling and sample processing, suitable for the study of their diversity by metabarcoding, was tested. Thirty-nine samples were collected from each tree, using mixed swab and whole thalli sampling methods, corresponding to specific algal communities: A. exolichenic; B. total community of lichen thalli; C. intrathallic; testing three surface sterilization methods; D. green algae from neighboring lichens; F. green algae inhabiting the dermosphere; G. green algae present in the phyllosphere. DNA isolation from 585 samples was performed using the Quick DNA Fecal/Soil Microbe Kit (ZymoResearch). To examine the diversity of selected algal communities, amplicons of the ITS2 rDNA region were prepared. Ligation of adapters necessary for sequencing and Illumina NovaSeq 2x250bp sequencing, was performed by an external company.

Results: PcoA analysis showed that it is impossible to distinguish completely the studied communities; they have a certain common part. Beta diversity and the Kruskal-Wallis test showed statistically significant differences between exolichenic and intrathallic communities. However, there were no significant differences between exolichenic communities and the dermosphere and phyllosphere, as well as the communities of green algae surrounding lichens. Analysis of the species composition of individual communities indicates that the same green algae species may occur in each community, but with varying abundance.

Conclusions: As the experiment did not allow for the description of unique exolichenic green algae communities, the hypothesis requires further verification. However, the obtained results may indicate that the method of exolichenic communities sampling using a swab may allow for the simultaneous assessment of the composition of the entire ecosystem.



Ancient Host-Associated Microbes obtained from Mammoth Remains

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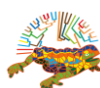
²⁰Natural History Museum, ²¹North-East Interdisciplinary Scientific Research Institute N.A. Shilo, Far East Branch, Russian Academy of Sciences, ²²Department of Archaeology and Classical Studies, Stockholm University, ²³Section for Computational and RNA Biology, Department of Biology, University of

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POSTER SESSION 1 - P01.073, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Ancient genomic studies have extensively explored human-microbial interactions, yet research on non-human animals remains limited. Here, we analyzed ancient microbial DNA in 488 mammoth samples, of which 359 are newly sequenced, spanning a time transect from the Holocene to over a million years ago. After accounting for contamination and conducting phylogenetic analyses, we identified six bacterial clades related to modern commensal or putative pathogenic species. These include *Streptococcus* from woolly mammoth teeth

on Wrangel Island, *Erysipelothrix* from a 1.1-million-year-old steppe mammoth, and *Pasteurella*-related bacteria that have previously been linked to African elephant deaths. Although additional complete genomes are required to fully evaluate the gene content of the ancient mammoth bacteria, the identification of potential virulence genes suggests that some microbes could have played a pathogenic role. Collectively, these findings highlight the significance of ancient DNA in investigating the microbial ecosystems of extinct animals.



Can my gut save me? Heatwaves, cloacal microbiome and survival of lesser kestrel nestlings (*Falco naumanni*)

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POSTER SESSION 1 - P01.113, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

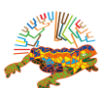
Background: Microbial communities, particularly those in the gut, play key roles in host metabolism, development, and immune function, often being referred to as a “second genome.” Commensal microbiota can affect thermal tolerance and survival in laboratory studies, but evidence from wild populations is still limited. This knowledge gap is particularly pertinent in the context of climate change, where rising temperatures may disrupt host-microbiome interactions during critical life stages. In cavity-nesting birds like the lesser kestrel (*Falco naumanni*), nestlings are constrained to the microclimatic extreme conditions of the nest which has an immense impact on development and given so can impact their microbiome and long-term fitness.

Materials and Methods: We examined the impact of high temperatures during a heatwave on the cloacal microbiome of lesser kestrel nestlings by means of a nest cooling experiment that created a natural gradient of nest temperatures. Nest boxes placed on roof terraces were either shaded or left unshaded, resulting in an average nest temperature difference of ~4°C (range: 40.0–53.7 °C) during extreme heat events. This setup allowed us to assess how variation in microclimatic conditions affects the nestling microbiome. DNA was extracted from cloacal swabs, and the full-length 16S rRNA gene was amplified and sequenced using Oxford Nanopore's 16S Barcoding Kit 24 V14 (SQK-16S114.24). Sequences were processed and taxonomically assigned at the genus level using the EPI2ME 16S workflow (v1.4.0). The resulting genus-level abundance table was imported into R (v4.5.0) for diversity and statistical analyses to

evaluate associations between microbial diversity, nest temperature, and other ecological variables.

Results: Whereas preliminary analyses revealed no significant differences in overall microbiome community composition when comparing shaded and unshaded nest boxes directly, linear mixed models revealed that microbial diversity increased significantly with rising nest temperatures, regardless of shading treatment (Shannon index: $p = 0.021$; Simpson index: $p = 0.047$). Importantly, nestlings that died before fledging had significantly lower microbial diversity, as measured by both indices ($p < 0.05$), suggesting a potential link between reduced gut microbiome diversity and increased mortality risk. Across all samples, the dominant bacterial phyla were Proteobacteria (notably Pseudomonadota) and Firmicutes (Bacillota), consistent with patterns reported in other cavity-nesting bird species.

Conclusion: Our findings suggest that high nest temperatures during heatwaves can shape the gut microbiome diversity of lesser kestrel nestlings, with increased temperatures associated with higher microbial diversity. Critically, lower gut microbiome diversity can be linked to higher mortality risk before fledging, suggesting that microbiome health may play an important role in nestling survival under extreme climatic conditions. These findings underscore the role of microclimatic conditions and host-microbiome interactions in shaping fitness outcomes in wild bird populations, offering new perspectives on how climate variation may influence developmental and ecological processes.



Characterization of Microbial Communities in Winter Ticks & Moose in Maine

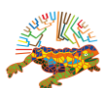
Miles M¹, Kantar L², Simpson B³, Ishaq S¹, Kamath P¹, De Urioste-Stone S¹

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POSTER SESSION 1 - P01.115, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Winter ticks (*Dermacentor albipictus*) are ectoparasites that complete their entire one-year life cycle on a single host, preferentially the North American moose (*Alces alces*). Infestations can lead to significant health issues in moose, including anemia, hair loss, and death, especially in calves. Microbial communities within winter ticks may play roles in pathogen transmission to moose, raising concerns about ecological impacts beyond direct physical effects on moose health. While this study does not explicitly test evolutionary hypotheses, it provides foundational data relevant to understanding host-parasite-microbiome interactions, which are integral to evolutionary dynamics in host-parasite systems. The goal of this study is to (1) characterize microbial communities in winter ticks parasitizing moose in Maine and (2) identify potential shared pathogens between ticks and their hosts. In January 2024, winter ticks and paired whole blood samples (n=93, respectively) were collected from moose calves (~8 months old) and a few adults during live captures in Maine's MDIFW District 4 and Penobscot Nation Territory. DNA was extracted from all samples and the 16S rRNA region was amplified via PCR prior to sequencing at the University of New Hampshire's Hubbard Center for Genome Studies using an Illumina NovaSeq platform to characterize microbial communities. Data processing included cutadapt trimming, filtering, and taxonomic assignment using the DADA2 pipeline. Taxonomic assignment was conducted on moose samples using the SILVA v138.2 database, while winter tick data are currently undergoing taxonomy assignment. Contaminant sequences were identified and removed based on frequency and prevalence in negative controls. The quality of all samples was high, with 8,753 amplicon sequence variants (ASVs) identified in moose blood samples and 20,339 ASVs in winter tick samples. Moose samples had higher read retention (87%) compared to ticks

(57%), but tick samples retained more ASVs (91%) after chimera removal (compared to 81% in moose samples). The most abundant phyla in moose blood included Pseudomonadota, Bacillota, and Actinomycetota, with notable genera such as *Eperythrozoon*, *Pseudomonas*, and *Anaplasma*. Further analyses of winter tick microbiomes will provide insights into microbes that could potentially be vectored by these ticks in moose. This study offers a comprehensive metagenomic analysis of microbial communities in winter ticks and moose hosts. The microbial profiles found in moose blood are consistent with those of other mammals, consisting of diverse taxa from environmental and host-associated phyla. Despite lower read counts, winter ticks exhibited greater microbial diversity, with more ASVs detected than in moose samples. Ongoing analyses will focus on refining data, standardizing species richness using rarefaction, and conducting statistical comparisons of alpha and beta diversity to explore differences in microbial structure. Comparative taxonomic analysis will also assess microbial overlap and identify potential shared pathogens between moose and winter ticks. These efforts will illuminate the functional roles of these microbial communities and their potential implications for moose health. A deeper understanding of the complex interactions among moose, ticks, and their associated microbes is essential for informing future wildlife disease management strategies. The findings of this research have the potential to significantly impact wildlife management, particularly in areas that may be affected by future climate change. Understanding how shifts in temperature and habitat could alter tick populations and pathogen transmission is crucial for predicting future health threats to moose and other wildlife. This study has the potential to enhance our knowledge of tick-borne diseases and could aid future mitigation strategies.



Comparative microbiome analyses reveal differences between wild and captive populations of the Montseny Brook Newt (*Calotriton arnoldi*)

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POSTER SESSION 1 - **P01.078**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The Montseny brook newt, *Calotriton arnoldi*, is a Critically Endangered amphibian species endemic to the Montseny Massif in Catalonia, Northeastern Spain. Due to population declines and threats to its natural habitat, an ex-situ breeding program was initiated in 2007. A key goal of the program is to ensure the survival of captive-bred individuals after reintroduction, which in amphibians heavily relies on the specimens' microbiome being capable of protecting them from environmental microorganisms, especially considering the global Chytridiomycosis pandemic caused by the fungi *Batrachochytrium dendrobatidis* (Bd) and *Batrachochytrium salamandrivorans* (Bsal). This study aims to characterize the microbiome of wild and captive specimens of *Calotriton arnoldi*, to identify differences in microbiome composition, and to determine their potential impact on captive-bred individuals upon reintroduction. Up to 7,438 ASVs (Amplicon Sequence Variants)

were identified from 138 samples from 21 and 61 wild and captive-bred individuals, respectively. Results indicate that wild populations from different subspecies have significantly different microbiome composition, as do wild and captive-bred groups from the same subspecies.

Additionally, dissimilarities in microbiome variability were only found within each subspecies, between wild and captive-bred groups. In terms of composition, certain bacteria were identified as potential markers for both wild and captive environments. Enhancing microbiome variability might improve the survival prospects of reintroduced specimens. Thus, subjecting captive specimens to a more natural environment while in captivity or a soft-release procedure could potentially mitigate the absence of exposure to other bacteria and potential pathogens from their native environment.



Development and Stability of the Equine Gut Microbiome: Microbial Succession in Foals and Seasonal Variation in Adult Horses at the Spanish Riding School

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POSTER SESSION 1 - **P01.108**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: The equine gut microbiome plays a central role in digestion, immune function, and overall well-being. Microbial fermentation in the hindgut enables the breakdown of fibrous plant material, producing vital nutrients and energy. Early in life, foals acquire their microbiota through maternal transfer and dietary changes that shape long-term microbial development. In adult horses, factors such as feeding routines, physical activity, and seasonal variations can also influence gut microbial composition. Understanding these microbial dynamics is essential to support the health and performance of horses of all ages and breeds.

Materials and Methods: This study combines two research efforts, both conducted on Lipizzaner horses at the Spanish Riding School in Vienna:

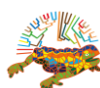
1. **Foal development study:** We followed 15 mare–foal pairs from birth to 12 weeks of age. Fecal samples were collected at several time points to monitor the development of the gut microbiota as the foals transition from milk to forage.
2. **Adult horse study:** In parallel, we are studying 20 adult stallions over a 12-month period to characterize their core gut microbiome and investigate how it responds to seasonal changes, dietary changes, training intensity and general health.

Microbial DNA was extracted from fecal samples, and the V4 region of the 16S rRNA gene was amplified and sequenced using Illumina technology. The data is analyzed to assess microbial diversity, community composition, and key factors influencing variation over time.

Results:

Initial sequencing and data processing have provided an overview of the microbial communities present in both foals and adult horses. Preliminary observations suggest dynamic shifts in gut microbiota during the early postnatal period, likely reflecting the influence of maternal microbiota and dietary changes. In adult horses, variation in microbial composition appears to correspond with temporal and environmental factors across the sampling period. Ongoing analyses aim to further characterize microbial patterns and identify potential core taxa shared across individuals and time points.

Conclusions: The findings from this study may guide future research and management strategies aimed at promoting a stable and resilient gut microbiome, supporting equine health and well-being from early development through adulthood.



Diet-microbiome associations in a wild Swedish carnivore

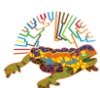
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POSTER SESSION 1 - P01.080, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The bacterial assembly of the gastrointestinal tracts in animals, i.e. the gut microbiota, provides the host with essential functions. Gut microbiota have been known to mediate host metabolism, train the immune system, digest food, and synthesize vitamins, yet it is still poorly studied in wild carnivore species. The wolverine (*Gulo gulo*) is an important Palearctic mustelid carnivore and scavenger, known for feeding on ungulate carcasses, hunting reindeer calves and food-stashing behavior. The Swedish population is considered vulnerable due to historic population declines and current low genetic diversity. Despite the wolverine's important ecological role and unique feeding behavior, its gut microbiota has never been studied in detail. Using a set of fecal samples collected for a national population survey, spanning the entire Swedish population, we sequenced the bacterial 16S rRNA gene in

over 400 wild individuals. The first aim is to characterize the wolverine gut microbiome composition, both spatially and temporally. We also plan to sequence the mitochondrial 12S rRNA gene of the same samples to characterize the host diet composition and perform diet-microbiome association analyses. Furthermore, to investigate how captivity impacts the gut microbiota of wolverines, we are sequencing samples from captive wolverines across fourteen European zoos. This large data set, comprising both diet and microbiome data from wild and captive individuals, will provide unique insights into geographical and environmental differences by taking advantage of existing samples collected for monitoring purposes. This research will be important to further our limited knowledge on wild and captive carnivore microbiota and diet to better protect the wolverine.



Diet-microbiome covariation across three giraffe species in a close-contact zone

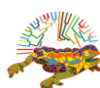
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POSTER SESSION 1 - P01.104, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The biodiverse group of ruminant mammals are entirely dependent on their gut microbiota to extract energy and nutrients from their foods, making these symbionts vital to survival. Because variation in wildlife diets can select for distinct communities of gut bacteria, different foraging choices can have both nutritional effects and other microbially-mediated effects on animal well-being. Despite the proliferation of studies focusing on host-microbiome interactions in recent decades, few prior studies have quantified the level and extent of diet-microbiome linkages in wildlife. Therefore, we used DNA metabarcoding to compare the diets and gut microbiomes of giraffes, the world's largest ruminant. We focused on three giraffe species—reticulated, northern, and Masai—that occur along a near-contact zone in equatorial Kenya. We found large differences in both the composition and diversity of diets and microbiomes within and among populations. However, contrary to expectations, we found very little evidence

for links between the composition of individual diets and their corresponding microbiomes. Instead, geographic proximity strongly predicted diet similarity whereas host-species identity strongly predicted microbiome composition. The lack of diet-microbiome linkages across these giraffe populations—coupled with the evidence that species differed strongly in diet and microbiome compositions—highlights the possibility that their history of ecological and evolutionary divergence has generated unique, species-specific gut microbiomes. Moreover, striking levels of diet variation were revealed among neighboring populations of giraffe from the same species, and thus baseline knowledge of their resource-use diversity could support ongoing efforts to geographically tailor management strategies aimed at conserving local food staples under environmental change.



Different drivers govern the fungi and bacteria components in the dung beetle microbiota

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POSTER SESSION 1 - **P01.077**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Many of the ecological services associated with coprophagy are provided by symbiotic microorganisms, namely the gut microbiota, the action of which may depend on host behaviour and ecology. The gut microbiota also influences dung beetle growth and development. The taxonomic composition of the gut microbiota is affected by various factors; of these, phylogeny plays a greater role than habitat or diet. The switching of the microbiota between syntopic sister species (e.g., *O. vacca/medius* and *O. taurus/illyricus*) causes significant problems. Intraspecific microbial variability is also very high due to vertical parental derivation. In *Onthophagus* the transmission of maternal microbiota occurs through the pedestal—a fecal secretion emitted by the female, on which she lays her eggs and the larvae feed after hatching. The present research examined the gut prokaryotes and fungi of wild adults collected from different types of dung located in distinct pastures. Our aim was to understand whether species identity affects the microbiota more than dung type and whether this conditioning applies equally to prokaryotes and fungi.

Materials and methods: Individuals of *Onthophagus fracticornis*, *O. medius* and *O. verticicornis* were collected from the western Alps (45.136° N, 7.296° E) from different types of dung (cow, sheep and donkey) located in distinct pastures. Following species confirmation by COI barcoding (in BOLD and ASAP), we selected 30 adults (15 males/15 females) of each species per dung type. After a 24h fast, the entire gut of each individual was dissected out and the prokaryotic (archaea and bacteria) and fungal components of the gut microbiota identified using the 16S ribosomal RNA (rRNA) gene and nuclear ribosomal ITS2 region, respectively. Statistical analyses were conducted for prokaryotes and

fungi separately, visualizing inter-species and inter-individual dissimilarities by means of non-metric multidimensional scaling (nMDS). Envfit analysis on the nMDS ordination determined which factor differentiated individuals the most.

Results: The relationships among the *Onthophagus* (*Paleonthophagus*) species were confirmed by phylogenetic analysis. The ordination plot of prokaryotes showed a clearer separation of points in the nMDS space, with individuals grouped by species rather than dung type. In the envfit analysis, species R² = 35.7% (p = 0.001). Dung type and sex accounted for 15 (p = 0.001) and 10.1% (p = 0.001), respectively. Conversely, the fungi ordination plot showed a clearer distribution of the points in the nMDS space, with individuals grouped by dung type rather than species. The R² value for dung type was highest (R² = 30.4%; p = 0.001), whereas species and sex accounted for 5.2 (p = 0.051) and 1.3 % (p = 0.282), respectively.

Conclusions: Microbial variation depends on phylogeny and diet. We found the gut microbiota composition to reflect two divergent trends: i) the prokaryotic component varied according to host species, with distinctions in even phylogenetically very close species, whereas it was constant within each species regardless of the dung type; ii) by contrast, the fungal component derived, at least in part, from the host's diet, being relatively constant within each dung type regardless of the species. Most prokaryotes of dung beetle microbiota were definitive symbionts, whereas many fungi were transient symbionts. These results support the notion that certain lineages of bacteria beneficial for host nutrition may be transmitted from mother to offspring, creating a kind of species-specific microbiota.



Early life assembly of the gut microbiome and its consequences in wild Soay sheep

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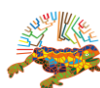
POSTER SESSION 1 - P01.112, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The gut microbiome (GM) exerts profound influences on host biology, shaping digestion, immune function, and pathogen defense. Early life represents a critical window for GM assembly, with long-lasting effects on host development. Because much of our existing knowledge regarding GM development comes from model systems, primates, and humans, more insight is needed into patterns of GM assembly across a diverse range of hosts. To address this gap, we investigated GM development across the first year of life in wild Soay sheep (*Ovis aries*), a natural population living on the island of Hirta in the St Kilda archipelago.

We sampled over 250 individuals longitudinally across five timepoints, generating >1000 samples spanning neonates (<1 month), lambs (2–12 months), and adults (>1 year). We observed striking differences in alpha and beta diversity, as well as microbial life history traits, across age classes. Neonatal communities were exceptionally low in diversity and highly dynamic, with composition shifting markedly during the first few months but remaining distinct from adults until 12 months of age.

Juvenile GM profiles featured high relative abundances of putative pathobionts, which declined as the microbiome stabilised. In contrast, taxa associated with fibre degradation and ruminal development increased significantly post-weaning, mirroring a dietary transition from milk to forage.

Lamb GMs also exhibited rapid microbial growth rates and enrichment of opportunistic taxa capable of exploiting naïve, nutrient-rich environments. Intriguingly, individuals with the fastest GM growth showed reduced overwinter survival, suggesting that these opportunistic taxa may out-compete other beneficial core GM taxa. Additionally, we detected higher microbiota similarity between lambs and their mothers compared to lambs and random ewes, indicating some maternal contribution to GM assembly. I will present dynamics of early-life GM development in wild Soay sheep, explore how these patterns intersect with host health and survival, and discuss the broader implications for understanding how GM assembly in early life may shape long-term outcomes in natural populations.



Egg and embryo microbiomes in the house sparrow (*Passer domesticus*): acquisition and consequences for embryo success

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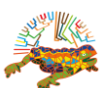
POSTER SESSION 1 - P01.102, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

All animals host microbial communities in and on their bodies, and recent studies have shown that variation in these microbiomes can have major effects on host phenotypes. Most notably, the vertebrate gut microbiome mediates critical host physiological processes, including digestion, development, immunity, and behaviour. How these gut microbes are initially acquired, however, is unclear. In oviparous vertebrates, some studies suggest that embryos develop in a sterile environment and gut microbiota are acquired after hatching. However, recent studies have begun to challenge this hypothesis, suggesting instead that gut microbes may be acquired in ovo, either via maternal transmission before laying or via trans-shell penetration of nest microbes. As such, our understanding of how oviparous vertebrates acquire their gut microbiome remains a topic of debate. Furthermore, whether the putative embryonic gut microbiome contribute to embryo success is unknown.

We used 16S rRNA sequencing and bacterial culturing to investigate the egg and embryo microbiomes of house sparrows (*Passer domesticus*). Specifically, we investigated the bacterial communities of eggs (yolk and albumen) immediately after lay, as well as embryos (yolk, albumen, and gut) that were

incubated either naturally in the nest or artificially under sterile conditions. We also sequenced the bacterial communities of the nest environment and female cloaca. Combined, these data allowed us to determine whether living bacteria colonise eggs or embryos prior to hatching, and explore the likely mechanisms of early-life microbiome acquisition. We also scored embryo survival and measured embryo body mass to investigate potential correlations between microbiota and embryo fitness proxies.

We successfully sequenced and cultured bacteria from eggs and embryos, which supports the existence of a living egg microbiome. Intriguingly, our data suggest that the nest environment has a relatively minor contribution to the microbiome of pre-hatch embryos. We also observed significant shifts in the yolk and albumen microbiomes between lay day eggs and embryos, even when eggs were incubated under sterile conditions. This finding suggests that the egg microbiome undergoes microbial succession during incubation. Taken together, our findings highlight the dynamic nature of the egg microbiome and suggest that bird microbiomes may accompany their hosts throughout their entire life cycles.



Ethanol preference as adaptive plasticity? Behavioral and microbiotic shifts in *Nosema*-infected honeybees

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POSTER SESSION 1 - P01.082, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

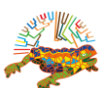
Background: Microbial communities within the gut play a central role in host physiology, influencing digestion, immunity, and behavior. In honeybees (*Apis mellifera*), the microsporidian parasite *Nosema ceranae* is a widespread, chronic, and often sublethal member of the gut microbiota. While typically framed as an invading pathogen, *Nosema* also functions as a background parasitic symbiont, routinely present at low abundance, that interacts with other gut microbes and host traits. It disrupts midgut function, leading to energetic stress and premature mortality, only under high infection levels. Here, we investigate whether *Nosema*-driven stress triggers behavioral plasticity in dietary preferences and whether such responses mitigate the costs of infection. We also assess how infection influences gut microbial composition, potentially contributing to broader microbiota-mediated host adaptation.

Materials and methods: We combined a meta-analysis of food intake in *Nosema*-infected vs. control bees with three additional laboratory experiments using caged bees of known age. In our experiments, individuals were assigned to control or infected groups, the latter receiving 100,000 *N. ceranae* spores in sucrose solution and the former receiving pure sucrose. Mortality was tracked for two weeks. In the meta-analysis and one experiment, we studied sucrose intake to test for compensatory feeding. In another experiment, bees were offered a choice between plain sucrose and ethanol-spiked solutions (0.5% or 1%) to assess infection-induced shifts in preference. In the third experiment, gut microbial communities were characterized by 16S rRNA gene metabarcoding (V3–V4 region), with analyses of alpha/beta diversity and differential abundance of sequence variants. Spore loads were quantified using qPCR to

confirm infection status and group differentiation.

Results: qPCR confirmed that infected bees had significantly higher spore loads than controls, validating the treatment groups in all experiments. Infection did not lead to increased total sucrose intake, suggesting no compensatory feeding response. However, infected bees showed a significantly higher preference for ethanol-spiked solutions, indicating an infection-linked behavioral shift. Moreover, while access to 1% ethanol reduced survival in control bees, this effect was less pronounced in infected bees, pointing to a potential buffering effect of ethanol under infection stress. Nevertheless, access to ethanol did not reduce parasite load. Gut microbiota composition did not differ strongly between groups, but several sequence variants varied in abundance, reflecting subtle changes of the microbial community in response to the developed infection.

Conclusions: Our results suggest that *Nosema* infection can trigger adaptive host plasticity via altered dietary behavior. This response, specifically the increased ethanol preference, may help bees mitigate the energetic and physiological costs of infection, even without stopping the proliferation of the parasite. Rather than dramatically reshaping the microbiota community, infection coincides with subtle shifts in microbial composition, indicating the complex interplay between pathogenic and other microbes in shaping host resilience. Our findings support the view that members of the gut community play a role in behavioral and physiological plasticity in hosts, even in the context of parasitism. More broadly, this work highlights how parasitic infections, as part of the microbial landscape, can shape context-dependent host adaptations.



Exploring the Effects of Habitat Modification on Rodent Gut Microbial Communities in the Yucatán Peninsula

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POSTER SESSION 1 - P01.087, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Habitat modification in natural forests is associated with changes in land use and resource availability, resulting in complex landscapes consisting of a heterogeneous mosaic of old-growth and secondary forest patches surrounded by different types of anthropogenic land cover (e.g., crops, cattle pastures, urban areas, roads). Wildlife tends to modify its behavior, diet, and dispersal patterns in response to such habitat modification. Environmental modifications also shape the assembly of microbial communities, their taxonomic, genetic, and functional diversity, altering the host-microbiome evolutionary relationships. The unevenness in animal microbial communities, known as dysbiosis, is related among others to changes in diet, pollution, urbanization, agriculture and grazing activities. Our objective was to determine the effects of habitat modification on the assembly of gut microbial communities in wild rodent species across landscapes with different modification levels, using an innovative network approach in a multiple host species system.

Materials and Methods: Our study encompasses three geographic regions in the Yucatán peninsula, Mexico, each representing different degrees of habitat modification: urban (Mérida), rural (Tizimín), and natural (Calakmul); within each region, we selected three sites representing a local habitat gradient from urbanized to conserved. To characterize regions and sites, we estimated the cover percentage of crops, urban areas, and bare soil using Sentinel images. We sampled the rodent community in each region and sequenced the 16S rRNA V4 gene in 67 rodent individuals of six rodent species. We estimated different indices, including alpha diversity within regions, beta diversity and dispersion, and evaluated differences among sites. We built co-occurrence networks per site and estimated the number of nodes, edges, and modules, and vulnerability. We associated these metrics with the landscape variables using linear

models and determined the microbial identity within each module.

Results: Microbial richness increased with habitat modification in the three regions, while the number of common species was higher in rural habitats. We identified significant bacterial structure between natural and urban sites in Tizimín and between natural and rural sites in Mérida. Regarding the landscape variables, we found that the abundance of microbial taxa was shaped mainly by bare soils. When comparing the global networks, results showed that Tizimín had a greater number of edges and nodes, while Mérida had the lowest; connectivity in Mérida lacked keystone taxa. Comparatively, network patterns per site showed that rural sites had more edges and nodes, while the number of modules increased with habitat modification gradient. These network properties were mainly associated with bare soils and crops. Furthermore, several bacterial families in different modules were associated with urban cover in Mérida and Tizimín rural sites.

Conclusions: Our findings show a common response to habitat modification across regions. Urban gut microbiota exhibits higher richness but greater homogeneity, which can have negative or positive consequences for fitness, depending on the particular microbes' function. Rural microbiota has a greater number of correlated taxa forming networks, likely due to the heterogeneous land cover types in these habitats. Additionally, habitat modification increases the number of modules or functional units of the microbial networks, while decreasing their connectivity and complexity. This indicates a strong functional selection or the random colonization of new species (dysbiosis), while simultaneously restricting the type of organisms that can colonize the host, leading to homogenization. Overall, microbiome network structure and composition are associated with urban surfaces and bare soils.



Exploring the role of *Zostera marina*'s microbiome composition under heat stress across life stages and populations

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POSTER SESSION 1 - **P01.096**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Seagrasses are the only flowering plants to have successfully adapted to marine environments. Approximately 70 species are distributed across all the continents' coasts, except Antarctica, providing a wide range of ecosystem services, including coastal protection, carbon sequestration, and habitat provisioning for diverse marine life. Despite their ecological importance, seagrasses face mounting threats from climate change, especially rising temperatures and nutrient pollution. While the effects of abiotic stressors on seagrass health have been the focus of many studies, the role of biotic interactions -- particularly those involving associated microbial communities -- and the interplay between biotic and abiotic stressors remain poorly understood. In particular, the composition and functional significance of the seagrass microbiome in response to environmental stressors are still largely unexplored.

We hypothesize that the composition and function of the seagrass microbiome contribute to host stress tolerance and local adaptation, as demonstrated in terrestrial plants. We expect the local microbial communities to play a particularly significant role in enhancing stress resilience during early life stages of seagrass.

Our model species is *Zostera marina*, one of the most extensively studied and widely distributed seagrass species, which thrives across a broad range of environmental gradients, including differing depths, salinities, and temperatures. In an acute heat stress experiment simulating summer heat waves, *Z. marina* plants from different Baltic Sea populations and three different life stages (6-month-old juveniles, 18-month-old-juveniles and fully-grown adults) were

examined. Both juvenile groups exhibited lower mortality while the youngest plants even showed a higher photosynthetic efficiency under heat stress, suggesting a more flexible and resilient metabolism compared to the fully-grown adults.

To investigate these dynamics, we extracted microbial DNA from plant parts of the different groups of the experiment, as well from the roots, the surrounding sediment, and ambient water. Amplicon sequencing was performed using a targeted set of 16S, 18S, and ITS primers to characterize key prokaryotic and eukaryotic microbial groups associated with *Z. marina*. A particular focus in the composition analyses is placed on diverse eukaryotic endophyte, including *Phytophthora* spp. and *Labyrinthula* spp., a group of protists consistently associated with *Z. marina*. Under stressful conditions, *Labyrinthula* spp. is hypothesized to become pathogenic, causing the so-called wasting disease -- a phenomenon responsible for a dramatic decline (~90%) of *Z. marina* populations along the Atlantic coast in the 1930s. Despite decades of research, the host-pathogen dynamics of this and other eukaryote-plant interactions remain poorly understood.

As climate change continues to intensify abiotic challenges for aquatic plants, understanding the complex interactions between seagrasses and their microbiomes is essential for predicting their resilience and informing conservation strategies. Insights from this study provide a deeper understanding of how microbial communities contribute to the persistence and possible evolutionary rescue of *Z. marina* under increasing environmental stress.



Facultative symbionts and climatic adaptation: testing thermal benefits of *Hamiltonella defensa* in aphids across latitudes

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POSTER SESSION 1 - P01.090, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Host-associated microbes can modify ecologically relevant traits and thus shape the evolution of their hosts. In aphids, facultative endosymbiotic bacteria may impose costs, such as a reduced lifespan, but can also provide a range of benefits, including protection from natural enemies and increased tolerance of heat stress.

The heritable endosymbiont *Hamiltonella defensa* is best known for rendering its aphid hosts resistant to parasitoid wasps. However, it has also been linked to enhanced thermal tolerance of its host. This effect might be relevant to explain seasonal as well as spatial associations between symbiont prevalence and temperature. In the black bean aphid (*Aphis fabae*), there is a striking pattern of decreasing *H. defensa* prevalence with increasing latitude. The southern range also harbors greater strain diversity in this symbiont. This raises the possibility that *H. defensa* may contribute to temperature adaptation in aphids.

To test this hypothesis, we compared the performance of multiple *A. fabae* lines, both infected and uninfected with *H. defensa*, originating from temperate (Germany) and Mediterranean (southern Italy) regions under cold (13 °C), intermediate (20 °C), and hot

(27 °C) conditions. Using a combination of antibiotic curing and artificial infection, we leveraged the clonal reproduction of aphids to separate the symbiont's effects from those of the host genotype. This allowed us to quantify the relative contributions of host and symbiont genotype to reproductive fitness across different temperatures.

The symbiont's effect varied among strains, across temperature, and also depended on the host genetic background, resulting in significant G×G×E interactions. Host genotypes showed the strongest and most predictable patterns of fitness across temperatures. Interestingly, aphid genotypes collected in southern Europe tended to perform better at the lowest temperature, which may be related to life-cycle differences. While northern populations overwinter as frost-resistant eggs, southern populations more often overwinter as adults, albeit at rather cool temperatures.

Overall, our findings suggest that the latitudinal distribution of *H. defensa* in *A. fabae* is unlikely to be driven by direct thermal benefits, pointing to a limited role of this symbiont in climate adaptation of aphids.



Fast-paced evolution of commensal bacteria facilitates insect host development under chemical stress

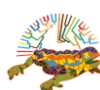
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POSTER SESSION 1 - **P01.079**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Human-made alterations to the environment are threatening insect biodiversity at an unprecedented rate. Anthropogenic chemicals such as insecticides and antibiotics are especially dangerous, as they can be released suddenly and in large quantities, leaving insects little time to adapt. Yet, insects may not be entirely defenseless. It has been proposed –but never experimentally confirmed– that the microbiome, i.e. the microbial communities associated with a host, could enable fast adaptation in response to stress. Using an experimental evolution experiment, we explored how environments polluted with an insecticide (imidacloprid) or an antibiotic (triclosan) could drive the evolution of *Gluconobacter oxydans*, a species naive to these chemicals and to *Drosophila*. With this setup, we also tested if these bacteria could associate with *Drosophila* larvae, and whether this association influenced their evolutionary trajectories compared to

bacteria evolved independently. After 10 fly generations of evolution (approx. 300 generations for bacteria), we observed faster development in bacteria-associated larvae, beyond that provided by bacteria at generation 0. This improvement was independent of larval presence, indicating bacterial adaptation to the fly food resource itself. Insecticide- or antibiotic-contaminated food caused developmental delays in larvae, but bacteria evolved on these chemicals fully rescued larval development in a compound-specific way. Consistent with this, we identified genetic changes in *G. oxydans* associated with the chemical they evolved with, highlighting potential targets for adaptation. Altogether, these findings suggest that the microbiome may indeed offer a mechanism for rapid host adaptation, although in *Drosophila* this appears to rely more on bacterial adaptation to environmental resources than on indirect effects involving the host.



Field realistic stressors alter the bee gut microbiome

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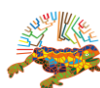
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POSTER SESSION 1 - P01.099, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Gut bacterial communities are known to regulate a variety of important functions in their animal hosts, including in the key pollinators bumblebees and honey bees. In bumblebees, the specialised gut microbiome ubiquitous across *Bombus* species is thought to contribute to host digestion, behaviour, and pathogen defence. Similar to honey bees, bumblebees have a simple but distinctive gut bacterial community. However, the interactions and potential sensitivity of these important gut microbiome functions to environmental stressors remains undetermined. We used controlled laboratory analyses to investigate which combinations of field realistic stressors may be influencing bee gut microbial communities. We exposed 40 bumblebee colonies, *Bombus terrestris*, to combinations of a pathogen, fungicide and pesticide commonly found in agricultural land, and assessed the gut bacterial communities of the bees using amplicon

sequencing. We show that some non-core taxa of the *Bombus* gut microbiome from the family Burkholderiaceae significantly differed in relative abundances in bees exposed to the field-realistic stressors when compared to controls. These findings highlight that some non-core gut microbiome taxa may be contributing to bumblebee adaptation to agricultural land, and are therefore important targets for functional analyses. In a key next step we have recently completed shot-gun sequencing of a unique set of 150 honey bee colonies from across the island of Ireland where pesticide and pathogen exposure has already been analysed for the same colonies. Both datasets are driving forward our understanding of bee host-gut microbiome interactions and the role of the gut microbiome in the evolutionary process, important for supporting pollinator resilience in the face of global change.



Genotype–environment interactions determine microbiota symbiosis in Syllidae polychaetes

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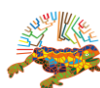
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POSTER SESSION 1 - **P01.103**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

While most animals possess species-specific microbiomes, the microbial symbionts associated with Syllidae polychaete annelids remain largely uncharacterized, despite their ecological significance as one of the main components of the oceanic benthos. In addition, the genetic and environmental factors that influence the structuring of microbial communities at the intraspecific level have been never studied in this family.

Thus, in order to dig into the host-specificity patterns of Syllidae species, we have analyzed the microbiome of different species collected in the Mediterranean Sea, Pacific and Atlantic Oceans, by amplicon sequencing of the microbial 16S rRNA gene. Furthermore, we have examined whether

geographic location or genetic structure of conspecific syllids mainly collected in the Canary Islands, influences their microbial assembly. As it was previously found in other marine invertebrates, host specificity at the genotypic level was observed in syllid species characterized by highly isolated populations (i.e., limited gene flow). In contrast, in species exhibiting high gene flow, microbial community composition appeared to be shaped more by geographic location than by host genetic divergence. Our findings indicate that, in syllid species exhibiting high population structure, host genetic clustering exerts a greater influence on microbial community composition than geographic location.



Gut Microbial Dynamics in Atlantic Salmon: A Natural Field-Based Common Garden Experiment on the Role of Host, Environment, and Stochastic Processes

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POSTER SESSION 1 - **P01.070**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Intestinal microbial communities are shaped by various ecological factors, including both environmental (E) and host-specific (G) influences (G×E). Few studies have assessed how these factors shape gut microbiota in Atlantic salmon populations in the wild. Here, we examine the roles of biotic, abiotic, and stochastic factors in shaping the gut microbiota of juvenile farmed, wild, and hybrid Atlantic salmon (*Salmo salar*) in a natural river system. Distinct microbial communities were found in the water, gut, and macroinvertebrates, with source tracking suggesting that most taxa in the salmon intestine originated from macroinvertebrates, a primary food source, rather than the water column. Seasonal shifts were observed in the contribution of macroinvertebrates to the gut microbiota, with the lowest contribution in winter and increased contributions in March and May, reflecting changes in food availability and fish appetite. Neutral modelling revealed that 86% of gut taxa were transient,

underscoring the dynamic nature of the gut microbiome. Additionally, host genetic background influenced microbial composition, with distinct assemblages observed between farmed and wild salmon. Our study highlights the complex interplay of deterministic and stochastic processes in shaping the gut microbiome of juvenile Atlantic salmon. The consumption of macroinvertebrates was identified as an important driver of microbial community composition, with seasonality influencing gut diversity. The high proportion of transient taxa emphasizes the need to distinguish between resident and transient microbes, especially in dynamic natural environments. Furthermore, the host's genetic background contributes to microbial variation, adding another layer of complexity to gut–microbe–environment interactions. These findings provide new insights into the assembly of the gut microbiome in wild salmon populations.



Heat-stressed *Aspergillus terreus* strain as a potential candidate for improving crop performance

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POSTER SESSION 1 - **P01.076**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

In this study, thermotolerant and plant growth-promoting endophytic fungi were isolated from *Citrullus colocynthis*, a wild melon native to arid desert environments. Among the isolates, DMF1, identified as *Aspergillus terreus*, demonstrated strong plant growth-promoting traits, including significantly elevated indole-3-acetic acid (IAA) production and phosphate solubilization when cultured under elevated temperatures (40+ °C), compared to control conditions (28 °C). Tomato seeds and seedlings inoculated with *A. terreus* DMF1 culture extract exposed to high temperature (40+ °C) exhibited significantly improved root length, chlorophyll content, water use efficiency and stomatal conductance compared to controls. RNA sequencing (RNA-seq) from DMF1 control (28 °C) and heat-stressed (40+ °C) was consistent with notable upregulation of Nitrilase and alkaline phosphatase genes under heat stress. Nitrilase is involved in IAA biosynthesis, contributing to auxin homeostasis and root development, while alkaline phosphatase plays a central role in phosphate mobilization and solubilization. These results provide molecular evidence that

DMF1 under elevated temperatures enhances specific transcriptional responses associated with plant growth regulation. Metabolite analysis revealed significant accumulation of cyclitol derivatives, including inositol and its phosphorylated forms. Inositol derivatives, particularly inositol polyphosphates and pyrophosphates, are recognized as multifunctional signaling molecules involved in phosphate storage, cellular stress responses, and hormone crosstalk. Notably, these metabolites are known to influence auxin perception and signal transduction, with potential downstream effects on root architecture, especially promoting primary root elongation under adverse conditions consistent with our in vivo findings. Our findings underscore the role of plant-endophyte interactions in helping the desert melon *Citrullus colocynthis* endure harsh desert conditions. They also highlight the potential of heat-stressed *Aspergillus terreus* DMF1—isolated from a naturally heat-adapted host—as a promising bioinoculant candidate for enhancing crop performance and sustainable agriculture under climate change.



Honey as a window into the honey bee microbiome : shedding light into diversity and host–environment interactions

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*POSTER SESSION 1 - **P01.084**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM*

Bees are composed of over 20000 species worldwide, playing a key role in pollination. Among them, only about ten species are capable of honey production, including the well-known *Apis mellifera*. Originally from Africa, the Middle East and Europe, this species includes about thirty subspecies, some of which have been extensively used and exported for the needs of the beekeeping sector. As a consequence, *Apis mellifera* is now present on every continent, except in Antarctica. Therefore, honey bees showed a remarkable capacity of adaptation and evolved successfully in a wide range of contrasting environments and climates. Furthermore, due to anthropic activities such as intensive agricultural practices (pesticides, insecticides) and climate change, honey bees have to face rapid changes and new emerging damaging diseases (*Varroa destructor* mite, small hive beetle...). Currently, these factors lead to an increased mortality of honey bees which will strongly impact the sustainability of the food production system and the overall ecosystems. To understand how bees have adapted to these varying environmental constraints and how they continue to face the new threats posed by global environmental change, we used honeys as an indirect proxy to characterize the composition and the role of their microbiomes. To this purpose, we analysed shotgun sequencing data coming from 121 honeys, corresponding to a large variety of geographic origins and subspecies of bees (including 107 honeys from all over Europe, 8 from Africa and 6 from other locations worldwide). These data were generated on 68 samples within our laboratory using MiSeq technology, while 53 samples come from open-access public databases, sequenced by a wider range of techniques (NextSeq, HiSeq, NovaSeq, BGISEQ). Overall, most samples show a low to medium depth (1.8 million reads on average for

117 samples), while a few were sequenced at greater depth (32 million reads on average for 4 samples). Among our samples, 22 honeys come from hives in France that have been furthermore monitored for their varroa mite infestation level. For the majority of our samples, we also know which plants were used for honey production, providing additional information for data interpretation.

To characterize the composition of honey's microbiota, we used a dedicated metagenomics pipeline (metaWGS) that allows us to access the taxonomic and functional diversity of the microbial communities. This flexible pipeline contains a succession of steps that allow the assembly, the binning and annotation of contigs as well as reconstruction of Metagenome Assembled Genomes (MAGs) at high quality. Through these analyses, we were able to assign a wide range of microorganisms, both eukaryotic and prokaryotic, that are present in honeys. In addition to the identification and provision of new genomic resources, we characterized the proportion of this microbial diversity using abundance tables, so as to be able to assess microbial variation in relation to the host genetic background, differences in climates or other environmental factors. Although my doctoral work is intended to be complemented with additional samples, particularly from more divergent bee species (including other species or even genera), the results already suggest variations in communities. We will particularly discuss the promise of genomic and metagenomic approaches applied to beehive products to help understand how microbiomes have co-evolved with their host, and how this evolutionary process can be associated with the long-term maintenance of bees in the natural and agricultural ecosystems in a global change context.



Human oral microbiome and its impact on dental caries across the last 7000 years in Anatolia

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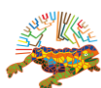
POSTER SESSION 1 - **P01.095**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Microbial communities play a vital role in host health, adaptation, and ecological flexibility, with the oral microbiome being one of the most diverse and dynamic ecosystems in humans. The composition of this microbiome is shaped by complex interactions between genetics, environmental factors, and diet. Among the health conditions deeply linked to oral microbiome dynamics, dental caries stands as one of the most common chronic diseases in human history. Its microbial origins lie in the breakdown of a balanced health-associated community into one dominated by acid-producing and acid-tolerant species capable of demineralizing tooth enamel — a transformation often driven by carbohydrate-rich diets and changing oral hygiene behaviors. As ancient human groups experienced local dietary shifts combined with broader cultural and demographic transformations, a similar complex interplay likely shaped the structure and diversity of their oral microbiomes across generations. Such transformations may also have altered microbial communities in ways that contributed to the emergence and persistence of caries. Southwest Eurasia may be particularly interesting as it underwent a transition to agriculture millennia earlier than many other regions. Despite the recognition of the oral microbiome's role in health and disease in modern studies, little is known about how these microbial communities evolved over long periods in response to the shifting ecological, dietary, and cultural environments of ancient populations in this region. Here, we investigate how long-term shifts in the human oral microbiome contributed to the increased prevalence of dental caries over a 7,000 year-long time span in Anatolia.

Materials and Methods: In this study, we examined the human oral microbiome associated with both dental health and caries in ancient Anatolian populations. We analyze ancient metagenomes from 56 individuals,

representing three distinct populations, spanning approximately 7,000 years from the Late Neolithic to the Medieval period. We screened 60 individuals from three distinct archaeological settlements based on their anthropometric characteristics and selected 56 individuals (16 Late Neolithic, 20 Middle Age, and 20 Medieval Period) with and without dental caries from each region. We sampled dental calculus from all available teeth per individual to maximize microbial DNA recovery and extracted DNA to prepare double-stranded genomic libraries for downstream analysis. We produced 10,000,000 paired-end sequencing reads per individual using Illumina Novaseq X platform. By analyzing sequencing reads, we reconstructed temporal microbial genomic profiles over the last 7,000 years. The bioinformatic pipeline aMeta was applied for taxonomic classification using k-mer signatures and alignment-based approaches, allowing us to confidently authenticate ancient DNA and accurately characterize the microbial community composition. Beyond taxonomic profiling, we calculated alpha and beta diversity metrics to assess within- and between-individual variation and track shifts in microbial community structure over time.

Results and Conclusions: Through this research, we provide a population-specific and time-resolved perspective on the dynamic relationship between the oral microbiome and human health. Our results highlight the evolutionary persistence of caries-associated microbial patterns and reveal how host health status, subsistence practices, and environmental factors collectively shaped the oral microbiome over millennia. This work further traces long-term changes in microbial community structure and explores how shifts in diet and lifestyle may have driven transitions between health-associated and caries-associated microbial profiles over thousands of years.



Impact of the presence of Varroa Mite on the Microbiota of *Apis mellifera* and *Bombus terrestris*

Borveto F¹, Wilfert L¹

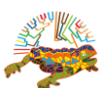
¹Institute Of Evolutionary Ecology And Conservation Genomics - Ulm University

POSTER SESSION 1 - P01.106, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Corbiculate bees such as Honey bees (*Apis mellifera*) and the Buff-tailed bumblebee (*Bombus terrestris*) harbor a consistent microbiome composed of relatively few species that are host specific. It has been shown that they play an important role in bee health not only in nutrition but also in the immune functions.

A. mellifera hives in Europe are commonly infested by the Varroa destructor mite, vector of several bee pathogens such as Deformed wing virus (DWV). The association of these two can lead to an increased viral load in foraging bees which are in regular contact with wild bumblebees.

In this study, we aim to explore the interactions between the honey bee specific parasite Varroa, viral prevalence and the microbiome of managed and wild bee populations. Our dataset consists of *A. mellifera* and *B. terrestris* samples from varroa infested and varroa free sites. Results from 16s rRNA sequencing reveal correlations between changes in the microbiome and the presence of pathogens in honey bees, namely *Pseudomonas*, *Wolbachia* and SBPV. While in bumblebees, we observe a strong effect of the presence of Varroa at the sampling site, and prevalence of DWV-B, despite the fact that Varroa is a honey bee specific parasite.



Integrative multi-omic analysis reveals oral microbiome-metabolome signatures of obesity

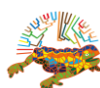
Jha A¹, Shibl A, Denekew T, Ahmad A, Abdelrazig S, Leonor C, Utenova L, Zhang G, AbdelBaqi M, Malla Y, Arshad M, Arnoux M, Drou N, Abdulle A, Ali R, Amin S, Idaghdour Y

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POSTER SESSION 1 - **P01.092**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Obesity is a major global health challenge and a leading risk factor for cardiometabolic disorders. The global surge in obesity, driven by industrialization and the widespread consumption of low-fiber, ultra-processed food, highlight an urgent need for deeper biological insights. While the gut microbiome has been studied in the context of obesity, the contribution of the oral microbiome—the second largest microbial ecosystem in the human body—remains largely underexplored. Here, we report findings from a deeply-phenotyped prospective-cohort of 628 Emirati adults, leveraging amplicon sequencing of mouthwash samples and multi-omics profiling and functional and metabolic activity analysis of 97 obese individuals and 95 matched controls, making this the most comprehensive multi-omics analysis of the oral microbiome. We identified significant differences in oral microbial diversity, composition, functional pathways, and metabolic profiles between obese and non-obese groups. Integrated multi-omics analysis of the 192 matched metagenomes and metabolome samples uncovered significant metabolic reprogramming and altered energy regulation in obesity. Specifically, the oral

microbiome of obese participants were enriched for the proinflammatory *Streptococcus parasanguinis* and *Actinomyces oris*, and the lactate-producing *Oribacterium sinus*. Many microbial pathways involved in dietary carbohydrate metabolism, histidine degradation, as well as the production of obesogenic biomolecules were also enriched in obese participants; however, B-vitamin and heme production pathways were depleted. Consequently, metabolites resulting from these pathways such as lactate, histidine derivatives, choline, uridine, and uracil were elevated in obesity. These consistent microbiome-metabolite shifts were strongly associated with prominent obesity-associated cardiometabolic markers, including serum triglycerides and alkaline phosphatases. These findings provide the most comprehensive insights into how disrupted microbial-metabolic cross-talk in the oral cavity may contribute to obesity and related cardiometabolic disease risk, underscoring the potential of targeting of oral microbiome-host interactions as a novel avenue for obesity prevention and intervention.



Intestinal tissue remodeling as a key adaptation to starvation in cave-dwelling fish

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POSTER SESSION 1 - **P01.110**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Food availability is one of the major evolutionary forces driving species evolution. In environments with limited nutrient availability—such as caves—cave-dwelling species have evolved unique adaptations to survive. The Mexican cavefish (*Astyanax mexicanus*) has repeatedly colonized subterranean environments, evolving metabolic and immune strategies that enable it to withstand long-term food deprivation. In contrast, its surface-dwelling ecotype displays relatively limited starvation tolerance. Given the gut's central role as the interface between the host and ingested nutrients, we investigated how intestinal tissue and its symbionts respond to prolonged starvation in both cavefish and surface fish. We combined ultrastructural morphological analysis, metagenomics, and single-cell transcriptomics to characterize the intestines of cavefish and surface fish under both starved and non-starved

conditions, at two and four months of complete food deprivation. Our results revealed that, under homeostatic conditions, cavefish exhibit higher activity of absorptive cells compared to surface fish, highlighting their enhanced metabolic capacity. Under starvation, surface fish show disruption of the mucosal landscape and impairments in intestinal integrity, whereas cavefish maintain intestinal structure even in the absence of food. We show that, even under controlled laboratory conditions, cavefish and surface fish harbor distinct microbial communities that are reassembled during starvation. These findings highlight cavefish as a powerful evolutionary model to investigate how dietary changes shape organismal evolution and establish them as a valuable natural system for studying prevalent human diseases that affect intestinal integrity.



Leveraging metagenomics to characterize the gut microbiome of caribou (*Rangifer tarandus*) for conservation.

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POSTER SESSION 1 - P01.093, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The gut microbiome is increasingly recognized as a key component of wildlife ecology and conservation, offering insights into host health, diet, adaptation, and responses to environmental change. This is especially important for threatened species like the caribou (*Rangifer tarandus*), whose herds in Western Canada are listed as species of special concern or threatened under the federal Canadian Species at Risk Act (SARA), and are declining due to habitat fragmentation, increased predation, and reduced connectivity. However, the microbial dimension of caribou health remains largely unexplored. In this study, we conducted the first large-scale metagenomic survey of caribou gut microbiota to investigate how host genetics, environmental context, and foraging behavior shape microbial diversity and function across herds. Using shallow shotgun sequencing, we analyzed 184 fecal samples from 21 caribou herds across British Columbia. We show that a sequencing depth of approximately 500,000 paired-end reads per sample is sufficient to resolve microbiome composition and functional potential with cost-effectiveness. Our bioinformatic analyses revealed a conserved core microbiome across herds, primarily composed of bacterial taxa associated with fiber degradation and host metabolic support. However, microbial community structure differed significantly between northern and southern herds, with southern, more

genetically isolated herds exhibiting markedly lower alpha diversity and distinct taxonomic profiles. We found that genetic differentiation better predicted microbiome composition than geographical distance. We discovered that smaller, inbred and isolated herds displayed markedly reduced microbiome diversity. Pathogen screening identified low-frequency but concerning occurrences of potential pathogens such as *Listeria monocytogenes* in certain herds, emphasizing the utility of microbiome data for wildlife health monitoring. Functionally, the microbiome exhibited high metabolic redundancy, but certain pathways were enriched in specific herds. Notably, southern herds displayed enhanced microbial capacities for starch degradation, an adaptation likely linked to a winter diet dominated by arboreal lichens. These localized functional adaptations highlight a possible link between microbial communities and caribou dietary specialization and underscore the potential for microbiome plasticity to buffer environmental challenges. By integrating microbiome data with host genomic and ecological information, this study provides a novel framework for understanding caribou health and adaptation at the microbial level. Our results highlight the gut microbiome as a sensitive biomarker for conservation applications to support decision-making around herd management, habitat restoration, and translocation strategies.



Linking phenotypic responses and advanced metagenomic techniques in *Daphnia magna* in response to combined environmental stresses

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POSTER SESSION 1 - P01.075, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Natural populations are increasingly exposed to a range of stressors including those linked to agricultural intensification, such as pesticide exposure as well as ecological pressures like altered predator–prey dynamics. These stressors often do not act alone but interact in complex ways, potentially amplifying their negative effects on freshwater ecosystems. The role of host-associated microbiomes in mediating stress tolerance is increasingly recognized, yet their contribution to responses in organisms under combined environmental stressors remains underexplored. This research investigates the microbiome's role in shaping phenotypic traits in *Daphnia magna*, an important freshwater organism, when exposed to both chlorpyrifos (an organophosphate insecticide) and fish kairomones (a predation cue). The experiment focuses on linking these phenotypic responses to shifts in microbiome composition and function, potentially influencing stress tolerance.

Daphnia clones were collected from twenty ponds with varying pesticide exposure and predation pressure histories. In the life history experiment the *Daphnia* clones were exposed to four treatments: control, chlorpyrifos, fish kairomones, and combined exposure, replicated three times. Age at maturity, reproductive output, body size, spine length and survival, were measured to assess phenotypic responses to individual and combined stressors.

This experiment aimed to determine an optimal pesticide and fish kairomones concentration based on one clone of each of four pond type, for a bigger follow-up study involving six clones from each of the twenty ponds. The goal was to identify a pesticide concentration that would provide a balance between survival and stress to detect meaningful differences, especially in combination with fish exposure. To achieve this, a range of pesticide concentrations was tested,

ranging from 0.1 to 1.4 µg/L, including combinations with fish. Based on the results, 0.2 µg/L chlorpyrifos is the optimal concentration together with 0.2 fish/L. Results also revealed significant clone-specific and stressor-dependent phenotypic responses. Across all clones, fish-exposed individuals produced larger clutches on average. Prior exposure to fish appears to provide a slight survival advantage, particularly under combined stressor conditions (pesticide + fish). In contrast, clones with no prior exposure to either stressor are more vulnerable to pesticide exposure, showing limited resilience.

While microbiome samples have not yet been collected, the follow-up phase of this study will involve *Daphnia* samples for metagenomics and microbial profiling at the end of the experiment. These microbiome analyses will help identify bacterial taxa and functional genes, such as those involved in pesticide degradation or stress resistance, that correlate with observed variation in phenotypic traits under combined environmental stressors. A novel aspect would be the application of adaptive Nanopore sequencing in metagenomics for selective microbial DNA enrichment, aimed at reducing host DNA contamination. Protocols are optimized using different DNA extraction kits and microbial DNA enrichment kits. These methods will be compared with traditional Nanopore sequencing workflows. By the time of the conference, preliminary results on the effectiveness of adaptive Nanopore sequencing will be available, which is expected to significantly improve microbial detection, particularly for low-abundance taxa.

By integrating phenotypic data with advanced metagenomic techniques, this study aims to provide new insights into the role of microbiomes in response to environmental stressors in *Daphnia magna*.



Metagenomic insights into the ageing gut microbiome in a natural population

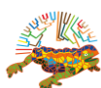
Lee C¹, Worsley S¹, Davies C¹, Silan E², Burke T³, Komdeur J⁴, Hildebrand F², Dugdale H⁴, Richardson D^{1,5}

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POSTER SESSION 1 - P01.072, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

While the influence of the gut microbiome (GM) on human health has been extensively researched, its impact on ageing and senescence in wild animal populations remains unclear. Indeed, studies addressing such questions in wild populations have yielded conflicting findings. Part of the problem is that acquiring the lifelong longitudinal data on individuals required to gain resolution on ageing effects is extremely challenging. I will address these limitations using a population of Seychelles warblers (*Acrocephalus sechellensis*) and analysing individual-based longitudinal metagenomic GM data to investigate how functional GM diversity, composition, and stability are associated with host age and senescence. Studies on humans suggest a complex interplay between GM and host senescence,

where age-related declines in physiological function appear to be linked to shifts in microbial composition and reduced GM stability. Therefore, we hypothesise that GM functionality will remain stable for most of adult life but differ significantly in late life, and especially just before death, as a result of host senescence. By using a combination of metagenomics and repeated sampling of individuals, we offer a more nuanced and potentially more accurate analysis compared to previous metabarcoding studies. In future work, this data will be analysed alongside host genomes to better understand how inbreeding (assessed through runs of homozygosity) interacts with the GM to influence host senescence trajectories.



Microbiome diversity in a successful urban bird

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POSTER SESSION 1 - P01.107, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: The expansion of urbanized areas severely transforms natural habitats and selective pressures faced by animals. However, some species function remarkably well within a man-made environment. A paragon example of an urban bird is the feral pigeon *Columba livia domestica*, which inhabits towns and cities around the globe, where it often thrives in extreme population densities. Feral pigeons show remarkable adaptations to urban landscape, but it remains unexplored how different components of this urban life modulate gut microbiome. The gut microbiome performs a number of roles, affecting host physiology, and consequently – its fitness. High level of sociality and frequent interactions with conspecifics are considered to be important factors leading to an increased gut microbiome diversity, due to facilitated horizontal transmission.

Materials and methods: We aimed to investigate how habitat urbanization and conspecific density can influence the taxonomic and functional diversity of the gut microbiome in a well-adapted urban bird, the feral pigeon. To address these questions, we sampled 200 adult feral pigeons across the gradients of habitat urbanization and pigeon population density in five large cities in Poland. Using the meta-barcoding approach based on the high-throughput 16S rRNA sequencing, we identified microbial assemblages in collected fecal samples. We calculated alpha (Shannon diversity and ASV richness) and beta (Jaccard distances) diversity indices. We also used the FAPROTAX database to assign functional profiles to the microbial taxa and to analyze

shifts in functional diversity of the gut microbiome. We tested the associations of habitat urbanization and pigeon population density gradients with microbiome diversity measures using GLMM models (alpha and functional diversity) and PERMANOVA (beta diversity).

Results: We found significant differences in taxonomic composition of pigeon gut microbiome between areas with different levels of urbanization and different host population density. We have not observed associations of pigeon population density with alpha microbiome diversity, however, we found a negative relationship between habitat urbanization score and ASV richness. When we investigated 10 most abundant functional groups, we found that none of them was associated with population density. Microbial groups associated with human and mammal gut, and, most importantly, human pathogens increased in relative abundance along urbanization gradient.

Conclusions: Our study was among the first addressing the links of bird sociality and group size with gut microbiome diversity in urban ecology context. Contrary to the expectations, we found little evidence of relationship between microbiome diversity and host population density. Interestingly, we detected changes in both ASV richness and functional diversity mediated by habitat urbanization. Especially, human pathogens were more abundant in more heavily urbanized habitats, which highlights the role of feral pigeons as pathogen vectors.



Rapid changes in the gut microbiome revealed by repeated short-term sampling in a captive guppy population

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*POSTER SESSION 1 - **P01.091**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM*

With the growing number of studies reporting associations between gut microbiome and diverse aspects of their hosts life, from physiology to behaviour and cognition, it becomes crucial to understand the dynamics of microbiome changes within individuals. While repeated sampling of individuals is often challenging, relying on a single sample - the most common practice - requires validation through evidence of gut microbiome stability. Here, we used a captive guppy population to assess short-term gut microbiome stability at the individual level, based on fecal samples collected over a two-week period from six fish. Bacterial taxa were sequenced and identified based on the 16S rRNA gene barcoding. We found substantial changes in microbial composition within few days,

mostly driven by rapid turnover of many rare taxa. At the same time, a 'core' microbiome could be identified for each fish, composed of very few abundant taxa. Interestingly, while the 'core' taxa mostly overlapped between individuals, there was significant inter-individual variance in their abundances. Taken together, these results suggest that even under stable environmental conditions the microbial composition of an animal's gut can undergo dynamic changes over short periods of time, without precluding substantial inter-individual variation in the gut microbiome. Thus, a careful analysis of the stability of a study organism's microbiome may help to determine the level of resolution required to conduct a reliable association study.



Reproductive Success Dependencies of Gut Microbiome: Insights from a Wild Population of Blue Tits

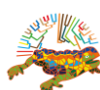
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*POSTER SESSION 1 - **P01.100**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM*

Gut microbiota composition and diversity are increasingly recognized as key factors influencing host fitness and reproductive outcomes in avian species. In this study, we investigated the relationship between gut microbiome characteristics and reproductive success in a wild population of blue tits (*Cyanistes caeruleus*) breeding on the island of Gotland, Sweden. Cloacal swabs were collected from adult individuals during two breeding seasons, with microbiome profiling performed via 16S rRNA sequencing. Alpha diversity metrics and beta diversity were analyzed in relation

to clutch size, fledgling success, and average nestlings' tarsus length (as a measure of offspring quality). The study provides novel evidence that gut microbiome may act as a proximate mechanism predicting reproductive success in passerines. A portion of broods included in our study also took part in a brood size manipulation experiment. Including this component in analyses indicates that the correlation between fitness components and microbiome composition metrics may be environmentally dependent.



Sex-Specific Evolution of Host-Parasite Interactions: The Interplay Between Host Genetics and Microbiome in the Immune Response in Trinidadian Guppies

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POSTER SESSION 1 - P01.069, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

1. Host responses to parasite infection involve several interacting systems. Host genetics determine much of the response, but it is increasingly clear that the host-associated microbiome also plays a role. Genetically determined systems and the microbiome can also interact; for example, the microbiome can modulate the host immune response, and vice versa. However, it remains unclear how such interactions between the host immune system and the microbiome may influence the host's overall response to parasites.

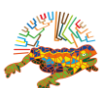
2. Host sex is an important source of variation in both immune response and the microbiome across systems, so interactions between them may also therefore differ between males and females, potentially explaining sex-specificity in response to parasites.

3. To investigate how host genetics and the microbiome interact to shape responses to parasites, we selected Trinidadian guppies (*Poecilia reticulata*) with low and high parasite resistance to the specialist ectoparasite *Gyrodactylus turnbulli*. Following experimental infection, we used the 30% of guppies with the lowest parasite

load to found the resistant line, the 30% with the highest load to found the susceptible line, and randomly chosen uninfected fish to found the control line. After 3-6 generations of breeding without parasites, we sampled the skin-associated microbiome and then infected fish from each line. We applied machine-learning to identify bacterial community types across lines and evaluated how selection line and community type explained variations in infection severity.

4. Our findings showed that among females, the resistant line had significantly lower infection severity, while the susceptible line had higher infection severity than other lines. Interestingly, females in the resistant line lost more mass per parasite, i.e., exhibited lower parasite tolerance, than in the control or susceptible lines. Among males, the susceptible line experienced higher infection severity than the other lines, but there was no difference between the resistant and control lines.

5. We found that the microbiome and host genetics independently explain similar variation in infection severity, highlighting the microbiome's active role in host-parasite interactions. Our results also indicate sex specificity in the evolution of host defenses.



Shaping Metabolism Through the Microbiome: Genetic Constraints on Host Plasticity in a Model of Obesity

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POSTER SESSION 1 - P01.071, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

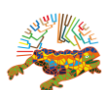
Background: Obesity and metabolic disorders pose major global public health challenges, with rising rates of metabolic syndrome across populations. Growing evidence indicates the gut microbiome acts as a critical mediator of host metabolic flexibility, influencing energy homeostasis and metabolic function. The db/db mouse model, featuring a leptin receptor gene mutation, displays hyperphagia, obesity, and hyperglycemia, providing an ideal system for studying how host genetic backgrounds interact with microbiome composition to shape metabolic outcomes. While correlations between gut microbiota and metabolic phenotypes are established, the mechanisms through which microbial communities enable metabolic adaptation remain poorly understood. Antibiotic treatment offers a valuable tool for gut microbiome modulation, allowing investigation of how microbial community restructuring impacts host metabolism. Early-life represents a critical developmental window when microbiome disruptions can fundamentally reprogram host metabolism, suggesting the microbiome's role in developmental programming.

Materials & Methods: We hypothesized that pre- and post-natal antibiotic treatment would restructure gut microbiome composition in db/db mice, enhancing metabolic resilience and improving outcomes. We bred db/b6 mice to produce homozygous db/db and heterozygous db/b6 offspring. Pregnant dams received either regular water or an antibiotic cocktail from pregnancy through offspring weaning until 8 weeks of age, creating eight experimental groups with sex and treatment variables. We monitored metabolic parameters and collected samples weekly

until sacrifice at 15 weeks. Analyses included energy harvesting efficiency measurements, inflammatory marker profiling, intestinal transcriptomics, and microbial community analysis.

Results: Our findings revealed that antibiotic-induced microbiome restructuring significantly enhanced metabolic health in homozygous db/db mice, demonstrating the microbiome's capacity to modify genetic predisposition. Antibiotic-treated homozygous mice exhibited improved metabolic parameters, such as reduced weight gain and better glucose homeostasis. Notably, heterozygous mice showed no significant response to identical treatment, highlighting how host genetic background constrains microbiome-mediated metabolic effects. Preliminary data suggest antibiotic treatment altered microbial community function, reducing energy extraction efficiency and shifting bacterial composition from predominantly Muribaculaceae and Sutterellaceae toward Lactobacillaceae and Bacteroidaceae in treated homozygous mice. These findings demonstrate the microbiome's role as a key mediator between host genetics and metabolism, capable of buffering genetic predisposition to obesity when modified during critical developmental windows.

Conclusion: This work provides compelling evidence that the gut microbiome contributes significantly to host metabolic plasticity in a genotype-dependent manner. Our findings highlight the potential for early-life microbial modulation to buffer genetic risk, and our ongoing research aims to identify specific microbial and metabolite mediators of these effects to inform targeted interventions for metabolic health.



Sustainable antimicrobial management in the burying beetle external microbiome

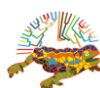
Duxbury S¹, Jardine M¹, Price T¹, Studholme D², Raymond B¹, Royle N¹

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POSTER SESSION 1 - P01.097, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Burying beetles (*Nicrophorus vespilloides*) exhibit a parental care system whereby they process small vertebrate carcasses as a nutritious resource to raise their larvae. This ephemeral resource must be defended against microbial degraders that colonise the carcass and surrounding soil. *N. vespilloides* is known to produce a diverse suite of around 40 narrow-spectrum antimicrobial peptides (AMPs) in secretions onto the carcass. These AMPs are predicted to suppress growth of target microbial competitors, whilst preserving beneficial microbes, originating from the carcass or beetle symbionts, that aid beetle reproduction and survival and larval development. The production of these AMPs in varying amounts and seeding of commensals may explain how resistance evolution in competitors has been avoided over millions of years of microbial management. Whilst there is some evidence for plasticity in AMPs production in response to perturbations in the levels and diversity of microbial competitors, the exact mechanisms have not been studied. To manipulate microbial competition on mouse carcasses, we set up three conditions in the laboratory using (i) antibiotic-treated or (ii) 'fresh' or (iii) 'smelly' carcasses, representing progressively advanced states of decomposition and decreasing resource quality. Beetle populations (lab-reared from the wild) were paired for reproduction and females were then exposed to these carcass types for their developmental cycle. Upon appearance of larvae, carcass 'pit' microbiomes were sampled and anal

secretions from female beetles were collected for quantitative proteomics. Reproductive output across treatments was measured. In line with predicted treatment effects on resource quality, offspring production (larval mass and brood size) decreased on 'smelly' carcasses, whilst antibiotic treatment improved reproductive output. Antibiotics reduced abundance of bacteria from the genus *Clostridium* in the managed microbiome. Across treatments, beetle processing of carcasses reduced abundance of bacteria from the *Lysinibacillus*, *Bacillus* and *Citrobacter* genera and increased bacterial abundance from the *Vagococcus* genus. Key AMPs associated with beetle immunity were quantified in the proteome of secretions (thaumatin-4 and c-lysozyme 2), with sustained production across the microbiome perturbation treatments. Proteome structure showed greater between-sample (beetle-family level) variance for secretions produced on 'smelly' carcasses as opposed to antibiotic-treated. This work shows that resource quality impacts beetle reproductive success; however, beetles can successfully manipulate their external microbiomes and plastically respond to perturbations in microbial competitors. Unlocking these strategies of resistance management will have broader implications both for understanding the role of plasticity in social evolution and in developing strategies to tackle the antibiotic resistance crisis in clinical settings.



Symbiotic communities of *Planococcus ficus* (vine mealybug) from two vineyards varieties

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POSTER SESSION 1 - **P01.109**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: The vine mealybug (*Planococcus ficus*, Hemiptera: Sternorrhyncha: Pseudococcidae) is a phloem-feeding insect and a major pest of grapevines (*Vitis vinifera*). It is the principal vector of Grapevine leafroll-associated virus 3 (GLRaV-3), the most widespread virus affecting vineyards. GLRaV-3 has a positive-sense RNA genome and is transmitted in a semipersistent manner. Notably, when *P. ficus* is reared on potato plants under laboratory conditions, it loses its ability to transmit GLRaV-3—an effect likely linked to both the absence of the natural grapevine host and changes in its gut symbiotic community.

Recent findings suggest that certain insect symbionts can interfere with the transmission of plant viruses, highlighting the importance of studying the gut microbiota of *P. ficus*. To investigate the biodiversity and dynamics of its gut symbionts, we sampled mealybugs from grapevines showing mild and severe GLRaV-3 symptoms and analyzed their associated microbial communities.

Materials and Methods: Mealybugs were collected from two vineyards (Dalton and Ein Zeitim), located 1.7 km apart and planted with different grapevine cultivars. From each vineyard, six samples were obtained from vines showing visible GLRaV-3 symptoms. Each sample included a grape bunch infested with mealybugs and five leaves from the same plant. A subset of mealybugs was transferred to virus-free grapevine plants in the laboratory, where they were allowed to reproduce for one generation.

Total DNA was extracted from surface-sterilized mealybugs from both field and laboratory colonies, and 16S rRNA amplicon sequencing was performed to assess bacterial community composition. In parallel, mealybugs were homogenized and plated on various culture media under aerobic and anaerobic conditions to isolate cultivable bacteria. Emerging colonies were identified through colony PCR and Sanger sequencing of the 16S rRNA gene. Presence of

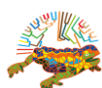
specific bacteria in leaf tissue was confirmed by PCR using species-specific primers.

Additionally, one pooled sample from each vineyard underwent Illumina whole-genome shotgun sequencing. Established pipelines were used to assemble and annotate bacterial metagenome-assembled genomes (MAGs), which were further analyzed for metabolic potential and virulence factors. Average Nucleotide Identity (ANI) and phylogenetic analyses were conducted on amplicons, cultured isolates, and MAGs to determine their evolutionary relationships.

Results: Mitochondrial cytochrome oxidase I sequences revealed distinct haplotypes between the two mealybug populations. Both carried the obligate symbionts *Tremblaya princeps* and *Moranella endobia*. Mealybugs from Dalton had high loads of *Serratia symbiotica*, while those from Ein Zeitim harbored an abundant but previously undescribed *Acetobacteraceae* species. Both bacteria were nearly absent after one laboratory generation, suggesting they are environmentally acquired. Their detection in grapevine leaves supports this hypothesis.

We successfully cultured *S. symbiotica*, later confirmed as strain CWBI-2.3 by ANI and phylogenetic analysis. The *Acetobacteraceae* isolate appears to represent a novel genus related to known bee gut symbionts. Comparative genomic analyses show both bacteria cluster with facultative insect symbionts and possess pathways for biotin synthesis, a vitamin absent from vine phloem.

Conclusions: Our preliminary results suggest that *S. symbiotica* and the novel *Acetobacteraceae* species are environmentally acquired, plant-associated bacteria capable of colonizing *P. ficus*. Their potential role in supplementing biotin suggests a fitness benefit to the insect. Moreover, the colonization capacity of *S. symbiotica* opens promising avenues for microbiome-based pest control strategies.



Testing for genetic differences in fitness across an environmental gradient using germ-free hosts to incorporate “local” microbiomes

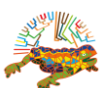
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POSTER SESSION 1 - P01.088, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Urbanisation is accelerating globally, causing adverse ecological conditions and disrupting ecosystem functioning. Urban ponds, increasingly implemented as nature-based solutions to mitigate climate-related impacts, are often exposed to harmful pollutants due to increased urban runoff. Zooplankton are typical inhabitants of urban ponds, and are key to the top-down control of algae, suppressing (toxic) algal blooms. As a result, disruption of the ecological functioning of zooplankton due to chemical pollution introduces risk for the ecological integrity of urban ponds. While recent work has demonstrated genetic adaptation in water flea *Daphnia magna* to urban stressors, including pollutants, the role of the microbiome in mediating tolerance has not been tested. Standard common garden experiments typically involve genotypes that have been bred in the laboratory and thus carry laboratory-shaped microbiomes. Here we use 18 germ-free *Daphnia* genotypes sourced from six ponds across a pronounced urbanization gradient in Berlin-Brandenburg area. These were exposed to field-collected

water – containing both the microbial communities and chemical signatures - of their own habitat or the habitat of other populations. This design allows us to test whether *Daphnia* genotypes differ in their fitness when exposed to different media and associated microbial communities and whether *Daphnia* genotype or medium-microbial community complex is the most important determinant of fitness. First analyses reveal that the origin of the environmental microbiome complex explains most of the variation in *Daphnia* fitness and mortality, whereas population origin had a much weaker effect. There was, however, a significant ‘population origin × environmental microbiome complex’ effect. Specifically, one highly urban population exhibited increased fitness in the sympatric condition, though this pattern was not a consistent across all populations. Our study underscores that both host genetics and combined effect of microbiome and environment, are key to understand adaptation in changing, urbanized environments.



The gut microbiota of hybrid birds: a possible role in speciation?

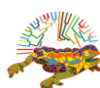
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POSTER SESSION 1 - **P01.089**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

In recent years, it has become increasingly clear that the gut microbiota of animals can be an important determinant of ecological adaptation, even having a significant impact on reproductive success and speciation. The symbiotic microbiome can shape speciation in different ways, and hybridisation provides a unique opportunity to learn more about this process by allowing the study of traits that are important for the build-up of reproductive isolation between genetically diverging populations under natural conditions. Incompatibilities in gut microbiota between hybridising species can lead to both pre-zygotic reproductive barriers by, for instance, influencing mate preferences, and post-zygotic reproductive barriers, by preventing successful hybridisation through negative effects on the hybrids' survival and fitness. In other cases, hybrids may acquire novel traits facilitated by their microbiota, potentially driving divergence and the formation of new species. However, studies of the gut microbiota of hybrids in vertebrates are scarce. As an initial step toward addressing this gap, this study aims to investigate the gut microbiota and diet of two closely related bird species, the pied (*Ficedula hypoleuca*) and collared (*F. albicollis*) flycatchers, and their hybrids. By examining the microbiome using amplicon sequencing of the bacterial

16S rRNA gene and the diet using the insect COI gene, we will explore possible differences in diversity and composition of the two traits among both parental species. Furthermore, we will compare the bacterial community and diet of the parental species with that of the hybrids to assess how distinct these traits are and where they lie in relation to the parental profiles. We hypothesise that the hybrid microbiota will be significantly different from both parental species, although potentially closer to their mothers. In terms of diet, given that both sexes contribute equally to feeding the offspring, we expect the diet composition of hybrids to be intermediate between the two parental species and to show correlations with the microbiome. Based on previous studies, we know that the *Ficedula* hybrids are sterile and experience higher, potentially dysfunctional, metabolic rates. Therefore, we plan to investigate the role of the gut microbiota in host metabolic function in a follow-up part of the study. These results will provide insights into the factors influencing the gut microbiome in the *Ficedula* genus, and how, in turn, microbial composition may influence metabolic function in hybrids, potentially contributing to post-zygotic reproductive barriers between the two species.



The Impacts of Feralization on the Horse Gut Microbiome

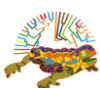
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*POSTER SESSION 1 - **P01.101**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM*

Feralization is a process that occurs when a population of domestic animals returns to wild conditions and can be thought of as a reversion of domestication. Because of the sudden return of both natural and sexual selection, feralization can result in large genetic, morphological, and behavioral changes in animals and their associated microbiota. Hence, when animals become feral, their microbiomes need to adapt as well. Despite intense focus on the microbial changes associated with domestication, the knowledge about how feralization affects the microbiome of animals is limited. Thus, feralization provides a unique opportunity to observe the microbiota responses to selection from a known (domesticated) standpoint. To investigate the impacts of feralization on the horse gut microbiome, fecal samples were collected from feral and domestic specimens of the horse breed Pottoka in Northern and Central Spain in 2023 and 2024. The gut microbiome was sequenced using metabarcoding of the v3-v4 region of the 16S rRNA bacterial gene. Variation in the gut microbiome composition

and diversity was evaluated across individuals and between feral and domestic horses. Furthermore, we investigated differences in the gut microbiota among feral populations, age classes, and sexes. The results indicate that feralization leads to a shift in gut microbiome composition, with lower microbial diversity in the feral horses compared to the domestic horses. These results may be explained by differences in diet, environment, and handling under feral and domestic conditions. We also observed prominent differences in the microbial communities between the feral populations, suggesting strong geographical and environmental influences on the gut microbiota, as well as differences between adults and juveniles. These results enable a better understanding of the process of feralization on the microbiota by providing insights into how the bacterial community in the gut can adapt to novel environments. This knowledge is crucial for understanding adaptation and evolution during the shift from artificial to natural and sexual selection.



The role of the chicken genome in shaping gut microbiota

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POSTER SESSION 1 - P01.111, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Animals harbor a diverse community of microbes within their intestinal tracts, known as the gut microbiota. It is known to impact host health and fitness, with essential roles related to the immune system, digestion and homeostasis. While numerous extrinsic and intrinsic factors are proven to impact gut microbiota composition, the role of host genetics is less clear. Emerging evidence points to an implication of immune and growth-related host genes and a potential for some microbes to be heritable. However, few studies have investigated the role of host genetics on shaping gut microbiota composition outside humans and model organisms. In this study, we used hologenomic data, to investigate associations between the host genome and

gut microbiota in two free-living island populations of feral chickens. We used 16s rRNA gene sequencing to characterize cecum microbiota variation and whole-genome sequencing to investigate host genomes from 269 feral chickens. Genome-Wide Association analyses (GWAS) were performed using microbiota alpha and beta diversity metrics, in addition to single microbial Amplicon Sequence Variants (ASVs). The results show associations between host genetic regions and microbiota phenotypes. This study will enable a better understanding of the role of the host genome in shaping gut microbiota composition, while also providing a broader insight on disentangling the complexity of host-microbiota interaction in nature.



The wild mammal oral microbiome is shaped by host diet and ecology

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POSTER SESSION 1 - P01.083, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Despite increasing interest, the microbiomes of wild animals remain largely undescribed, especially for body sites other than the gut. Shaping these communities on a macroevolutionary time scale, two processes are commonly referenced: a) host-microbiome co-diversification (through vertical transmission) and b) environmental filtering (selection due to environmental conditions). However, their relative importance remains intensely debated, and further study of wild microbiomes is hindered by sampling difficulties. Here, we use dental calculus from museum specimens to explore the evolution of the oral microbiome in wild mammals. Dental calculus is a mineralized form of dental plaque that preserves the DNA of the oral microbiota, allowing its exploration long after the host's death. While prevalent among mammals, dental calculus has mostly been studied in humans and other primates. Here, we expand the analyses to

oral microbiome of 32 species (the majority newly sequenced), representing a large part of mammalian phylogenetic, dietary, and ecological diversity (including marine, semi-aquatic, and terrestrial species). We find that both host diet and higher-level host taxonomy (at the order level) affect what taxa are found in the oral microbiome, but host diet also affects the gene content and metabolic potential of these communities. A more fine-grained analysis (comparing the phylogeny of host species to that of individual microbial genomes) reveals only a moderate co-diversification signal for certain studied microbial clades. We therefore propose that the oral microbiome community is shaped primarily by the environmental conditions experienced by the host (as those are formed by host diet and genetic factors) and to a lesser extent by co-diversification due to vertical transmission.



What maintains the diversity of the gut microbiome in termite populations?

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POSTER SESSION 1 - P01.098, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

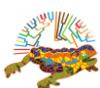
For the microbiome to drive host adaptation, it is essential that the variation in host phenotypes mediated by the microbiome is heritable. Vertical transmission - the transfer of microbes from parent to offspring - is probably the most straightforward way to ensure such heritability in populations. However, vertical transmission is often imperfect, meaning that offspring possess only a subset of the parental microbial taxa. Nevertheless, many factors can maintain microbiome diversity across host generations, including natural selection, horizontal transmission, and host population structure and mating system. Despite their importance, these factors are difficult to study in field host populations and remain poorly understood.

We aim to understand how the diversity of gut microbial community of termites is maintained in populations. Termites are social insects that live in colonies, typically consisting of a royal couple (a queen and a king) and its offspring. The termite hindgut harbours a diverse community that enables digestion of dead plant material such as wood. Termite colonies are founded by a couple of winged reproductives called 'alates', which differentiate and fly away from two unrelated parental colonies. Alates play a crucial role in vertical transmission by harbouring gut microbes from their parental colony and passing them on to their offspring. Interestingly, because colony foundation requires both a queen and a king, termites may be one of the few species in which symbionts are transmitted by both parents (biparental transmission).

We recently analysed the gut microbial community in two populations of the termite *Reticulitermes grassei*. By sequencing amplicons of 18S and 16S rRNA genes, we characterised the community diversity within 24 colonies and estimated the vertical transmission rate of each microbial taxa by examining the gut

community of 336 swarming alates of both sexes.

Overall, the results show that vertical transmission of microbial taxa is imperfect; many alates harboured only a subset of the microbial taxa present in their parent colony. We then used simulation models to investigate factors that might compensate for the loss of diversity caused by imperfect vertical transmission. First, we tested the effect of an unbiased biparental transmission on community diversity within colonies. As new colonies are founded by two alates swarming from different parental colonies, they could receive different and complementary microbial taxa from their parents, potentially increasing diversity. Our simulation models using the alate data show that biparental transmission alone is not sufficient to predict the observed within-colony diversity in populations. We then tested three additional hypotheses. The first, called 'disassortative mating system', suggests that alate males and females with dissimilar and complementary gut communities mate more frequently than would be expected from random mating in populations. The second hypothesis, called 'colony level selection', suggests that incipient colonies with too low levels of community diversity die out rapidly in the population and are counterselected. The third hypothesis, called 'mixed-mode transmission', considers that termite colonies acquire symbionts not only from the couple of alates but also from the environment (horizontal transmission). For each model, parameter values that allow for the maintenance of community diversity in the population, and their relevance in the light of termite population biology will be discussed.



A bloody old habit: Conservation of gut-associated bacteria across blood-feeding leeches

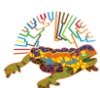
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MEETING ROOM 116, August 18, 2025, 2:00 PM - 5:00 PM

Leeches (Euhirudinea) are a clade of segmented worms (Annelida) that groups well over 800 known species. Haematophagy, or blood feeding, is an evolutionary innovation that lies at their evolutionary origin: Leeches likely evolved by a shift in feeding habit of their ancestor from a freshwater ectocommensal to a bloodsucking fish parasite. Present-day leech species are either specialised on feeding on vertebrate blood or have secondarily become predators. An exclusive blood diet poses challenges such as facing a B-vitamin deficiency plus an accumulation of iron and urea, resulting from haemolysis and protein catabolism. However, leeches are themselves hosts to symbiotic microorganisms that can help the leech compensate these challenges. For example, while anticoagulant proteins are encoded in the leech genome, B-vitamin supplementation is carried out by well-described endosymbiotic bacteria. Previous studies have shown that core microbes associated to the digestive system (which are body-site specific) of two so-called medicinal leeches belong to the same higher taxonomic groups. Therefore, in this study we asked whether the associated microbes

of distantly related haematophagous leeches are conserved in terms of not only taxonomy, but potential metabolic functions. For this purpose, we have produced high-quality reference genomes as well as retrieved high-quality sequencing data from a number of leech species. Using this strategy, we reconstructed closed and lowly fragmented genomes for most recognised core-microbes of leeches. We found that a fraction of these symbionts show clear signs of genome reduction, suggesting a long history of vertical transmission. Moreover, these symbionts are closely related and often monophyletic, suggesting a long history of association with leeches. Finally, through genome-based metabolic inference, we identified conserved metabolic pathways in the symbiont genomes that may play an essential role in maintaining an haematophagous lifestyle. In summary, we produced the first genome-resolved catalogue of leech-associated bacteria, and through an integrative analysis, propose a likely ancestral role of the leech's core microbiota in not only the maintenance of a blood-based diet, but also in the evolution of their blood-feeding habit.



Are the root microbiome and plant phenotypic traits affected when a wild perennial is transplanted across contrasting habitats?

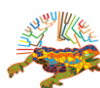
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Knowledge of how plant microbiomes are shaped and how the microbiota interact with their hosts are mostly based on studies from a few model species or crops. Less is known for wild non-model species, in particular how the microbiome could be interlinked with plant phenotypic plasticity across habitats. We have previously found that the root microbiota of the perennial vine *Solanum dulcamara*, a wild relative to potato with extreme phenotypic plasticity, differ among habitats ranging from dry beaches to wet forests but still possesses a similar core set of bacterial and fungi taxa. Here our overall aim was to understand how the root microbiome and plant phenotypic traits were affected by transplanting *S. dulcamara* from the three contrasting habitats beach, forest

and rural to a joint site. Using amplicon sequencing, we characterized root communities of bacteria and fungi in the natural populations and at the transplant site. We also studied phenotypic plant traits. We found that transplanted plants became very similar at the joint site compared to growing in their natural population in terms of growth, reproduction and nutrient status. The microbiota also become more similar, in particular the fungal communities, while the bacterial communities still showed some differences. While our results confirm that *S. dulcamara* is highly plastic and can easily adapt its growth to contrasting environments, it appears that the change in the microbial communities could be slower than that seen for phenotypic plant traits.



Bat gut microbiomes recapitulate host phylogeny and foraging niches

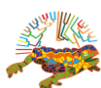
Melville D¹, Brändel S^{1,2,3}, Nolzen A¹, Kümmerle C¹, Geiger S¹, Wilhelm K¹, Tschapka M^{1,3}, Page R³, Wasimuddin W^{4,5}, Sommer S¹

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The role gut microbiota play in bat ecology and evolution remains a feverishly debated topic. Rapid gut transit times, low bacterial loads and weak signals of phylosymbiosis argue for a limited functional contribution, but the readiness with which bat gut microbial communities are affected by changes in diet, infections, or habitat disturbance paints a different picture. Here we re-examine evidence of phylosymbiosis and host specificity as well as the synchronicity of host foraging and microbiome changes in the so far largest collection of gut microbiomes (16S-rRNA taxonomic and PICRUSt2 inferred functional data; n=1211) from 16 bat species sampled along the Soberanía National Park in Panama. We detect clear signs of phylosymbiosis, which are strongly linked to diversification along foraging niches of phyllostomid bats. Evidence for co-

speciation was accompanied by moderate levels of co-diversification within foraging guilds sharing core ASVs, and taxonomic as well as functional specificity was highest for closely related hosts with shared foraging niche. Finally, we compared samples collected early in the night to those gathered later within the two bat species with the largest sample sizes to assess whether functionally relevant ASVs underwent temporal shifts from a starved to fed microbiome as evidence for co-synchronised biological rhythms of host and its microbiota. A shift was detectable and driven mainly by ASVs supporting host metabolic functions. Our work proposes that the evolved cross-talk between bats and the gut microbial symbionts is likely tied in with their diet specialisation and evolutionary diversification.



Behavioural Manipulation by Parasitic Nematodes is Associated with Microbiome Composition

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MEETING ROOM 116, August 18, 2025, 10:30 AM - 12:30 PM

Helminth parasites have complex life cycles, often going through a combination of free-living and parasitic stages in different hosts and habitats to achieve maturity and reproduction. Therefore, each helminth life stage has complex adaptations that enable them to reach the host or habitat required by their next stage. One of these adaptations is the remarkable ability of some helminths to induce water-seeking behaviour in their invertebrate hosts. For example, the adult mermithid nematode *Thaumamermis zealandica* must emerge from its host in water-saturated sand in order to reproduce. Previous work has shown that these parasites may induce their sandhopper hosts to bury deeper towards wet sand when compared to uninfected sandhoppers. However, there is variation in the level of behavioural change induced by conspecific parasites in their hosts, and variations in the microbiome have been proposed as a potential explanation for differences in behavioural manipulation, representing a form of microbiome-mediated plasticity.

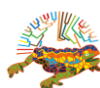
We conducted an experimental test of the effect of the microbiome of *T. zealandica* parasites and of their sandhopper hosts on the degree of host behavioural alteration in a humidity gradient.

Sandhoppers were placed in tubes filled with vertical layers of wet sand at the bottom, humid sand in the middle, and drier sand on top. They were left under natural light and temperature overnight, following which they were trapped within the sections in which they were buried. Upon dissection, any infecting parasites, as well as haemolymph of infected and uninfected sandhoppers

were taken for microbiome characterisation with the V4 region of the 16S rRNA gene. In addition, all sandhoppers and worms were measured to account for potential effects of size (as a proxy for maturity) on microbial community composition.

The composition of the parasites' microbiota was significantly correlated with the depth at which their sandhopper host was found, regardless of the size of parasites and sandhoppers. Interestingly, no difference was observed between the sandhoppers' microbiota at different depths or between infected and uninfected sandhoppers. The microbiota of parasites, sandhoppers and of the surrounding sand was significantly different, with many microbial taxa found in parasites not detected in the sandhoppers or the sand, supporting that mermithid parasites have a microbiome of their own. The finding that the parasites' microbiota, and not the hosts', is associated with host burying depth supports the hypothesis that parasites' microbiomes may indeed play a role in the parasites' ability to manipulate their hosts' behaviour.

Evolutionarily, *T. zealandica* parasites that manipulate the behaviour of their hosts may have a fitness advantage over their non-manipulative conspecifics, since they are able to emerge in a suitable environment for reproduction (wet sand). Our finding that manipulative parasites have a different microbiome from non-manipulative parasites suggests that at least some components of the parasites' microbiomes may play a role in parasite-host interactions.



Beyond Genes: Modeling Microbiome-Mediated Responses to Selection

Week B^{1,2}

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MEETING ROOM 116, August 18, 2025, 2:00 PM - 5:00 PM

Microbiomes can shape host traits and fitness, yet we still lack a general framework for predicting when such microbial effects influence host evolution. In this talk, I present an extension of theoretical quantitative genetics that incorporates microbes—alongside host alleles—as contributors to trait variation, and propose to decompose microbiomes by sources of acquisition. Building on this decomposition, I introduce microbial analogues of additive genetic variance and narrow- and broad-sense heritability, and show how transmission mode governs whether microbial effects translate into a response to

selection. Using simulation models, I demonstrate that microbes acquired non-vertically can still mediate host adaptation if their acquisition is shaped by selection. These results suggest that standard approaches in microbiome-wide association studies may yield biased estimates of evolutionary relevance unless microbial transmission is considered. This framework not only generalizes core tools in quantitative genetics, but also offers a practical foundation for identifying which microbiome components matter for predicting trait change across generations.



Can the gut microbiome mediate long-lasting effects of early-life environmental conditions on adult thermal physiology?

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MEETING ROOM 116, August 18, 2025, 2:00 PM - 5:00 PM

The ability to cope with temperature variation is a key challenge for all organisms. Individuals need to be able to quickly respond and adapt to increasingly unpredictable environmental variation across their lifespan. One promising mechanism that enables the host to adapt quickly to rapid environmental perturbations is the gut microbiome (GM). We recently showed in birds how short-term variation in environmental temperature alters the adult GM, this change having causal effects on host thermoregulatory performance. Thus, the GM can act as a source for phenotypic plasticity. In addition to this, the host-associated GM has further been suggested to help mediate developmental plasticity, but data from wild populations is scarce. Therefore, to explore whether the GM can act as a mediator for host developmental plasticity we studied the long-lasting effects of the early-life environment on adult thermal physiology using wild great tits (*Parus major*)

as the study system. During the main growth period, wild nestlings were either unmanipulated to serve as control individuals, exposed to altered temperatures to establish the effects of early-life thermal environment, or administered antibiotics to directly modify GM, and test whether the GM is mediating effects on host thermal physiology. First, growth and survival until fledgling was monitored in all individuals (n=528). Second, a subset of individuals (n=114) were reared in outdoor aviaries until adulthood in late winter, and various metabolic and thermal traits (including whole animal metabolic rate, cold tolerance, and mitochondria function) were measured. By conducting manipulative experiments in the wild, combined with common rearing under similar conditions this study can shed light on the role the GM can play in mediating the effects of early-life thermal environment on thermoregulation into adulthood.



Elucidating interactions of the microbiome and host genomics during population decline through a temporal hologenomics approach

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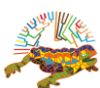
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Population decline is exacerbated by a myriad of factors, including habitat degradation, climate change, competition and predation from invasive species, and loss of genetic diversity. Genomic depauperation is of particular concern, as low diversity and inbreeding not only affects population fitness and disease susceptibility, but may also impact a species' capacity to adapt to novel or changing environments. While the importance of genetic diversity has been recognised for several decades, we are only just beginning to gain insight into the crucial roles that microorganisms play in species conservation. Symbiotic microbiomes play a central role in many biological processes and are intrinsically linked to host health. The composition of host-associated microbiomes is mediated by host genetics, which is particularly relevant to wild systems in the face of global climate change and rapid population declines. It is thus crucial to understand how changes in genetic diversity impact host-microbiome interactions. Achieving this goal in wild populations requires samples that span decades-centuries of population declines, yet host

associated microbiomes often do not preserve after host death. To address this significant knowledge gap, we utilise dental calculus, the calcified oral microbiome, from museum-preserved specimens of Scandinavian brown bears that experienced a dramatic population bottleneck over the course of the last 200 years. We observed the prevalence of oral disease increased over time, even after population recovery ($p < 0.001$). We then characterised the temporal diversity and function of oral microbiomes in healthy and diseased brown bears to define dysbiotic profiles directly associated with caries and diseased individuals. Moreover, we also explored the influence of a disease phenotype on microbial communities sampled from non-affected teeth to characterise spatial microbiome variation in diseased bears. Understanding the interplay between symbiotic microbial communities and host genetic diversity, i.e. hologenomics, is necessary for predicting the impact of host population decline from a holistic perspective, encompassing the consequences of altered microbiome composition and function on overall host health.



Functional implications of changes in the rumen microbiome

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The arctic climate is changing faster than anywhere else on the planet, most notably in terms of warming. For arctic herbivores such as muskox (*Ovibos moschatus*), climate change also means changes in the quality of their diet. As the Arctic continues to warm, plant nitrogen is expected to decline by as much as 14% in the key foraging areas of arctic herbivores. Genetic variability of the muskox is low due to multiple population bottlenecks over time, and populations may be poorly equipped to deal adapt to rapid environmental change. The extent to which the rumen and gut microbiome may either buffer or exacerbate the effects of such climate-induced declines in plant diet quality is currently unknown.

We used a controlled, longitudinal experiment on a captive muskox herd manipulating diet quality with repeated sampling over a 12-week period to understand the functional implications of rumen microbiome changes for ungulate energy acquisition. We combined metabarcoding, metabolomics, and lab-based digestion experiments to assess

rumen microbiome function. Based on well-understood metabolic pathways, quantification of the metabolome allows for the evaluation of the function of the metabolism while digestion capacity provides a direct measure of rumen microbial function.

We observed rapid microbial responses, with changes in diversity patterns being reflected in the metabolic concentrations. We show that declining forage quality increased microbiome diversity, leading to a wider range of metabolic capabilities within the gut and increased digestive capacity. This suggests a strong functional link between the gut microbial community structure and the metabolic landscape enabling hosts to buffer forage quality declines and maintain adaptive capacity. By linking microbiome composition and function, we elucidate the rumen microbiome's role in the adaptation of high-latitude ungulates to rapid environmental change, a crucial factor for predicting their future viability in a warming Arctic.



How the environment, genome, and microbiome influence niche width in spider mites

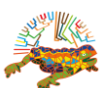
Bisschop K^{1,2,3}, Uller T³, Feiner N^{3,4}, Zélé F⁵, Wybouw N², Bonte D², Decaestecker E¹

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Adaptation to global change depends not only on genetic adaptation and phenotypic plasticity, but also on host-associated microbiota, which may influence nutrient uptake and detoxification, ultimately enhancing host resilience and expanding ecological niches. What drives some individuals to specialise while others remain generalists? To explore this, we studied the two-spotted spider mite (*Tetranychus urticae*), a herbivore with a host range

spanning over 1000 plant species. Combining field sampling in Southern France with lab experiments on isofemale lines, we measured performance across five host plants to identify niche width, and used whole-genome resequencing to uncover genetic and microbial contributions to niche variation. This work sheds light on the interplay of environment, genetics, and microbiomes in shaping adaptive potential and niche width.



Influence of Gut Microbiota Perturbation on Obligate Endosymbiont Abundance and Host Phenotype in the German Cockroach (*Blattella germanica*)

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The German cockroach (*Blattella germanica*) maintains a dual symbiotic system comprising the obligate intracellular endosymbiont *Blattabacterium*—housed within specialised bacteriocytes proximal to the urocytes—and a complex gut microbiota. While *Blattabacterium* is integral to host nitrogen metabolism, including uric acid degradation and essential nutrient biosynthesis, the extent to which its abundance and function are influenced by the surrounding gut microbial community remains insufficiently characterised. In this study, we investigated how experimental manipulation of the gut microbiota impacts *Blattabacterium* abundance and host phenotype. Our study aims to offer new insights into the multi-level host-microbiota interactions of this important hemimetabolous pest insect species. Experimental cohorts included a conventionally reared group (NS), alongside four sterile or semi-sterile treatment groups: (i) sustained antibiotic exposure post-sterilisation (AB), (ii) two-week antibiotic regimen (AS), (iii) two-week antibiotic

treatment followed by re-inoculation with a conventional gut suspension (GS), and (iv) re-inoculation with both gut suspension and faeces (GSf). Quantitative PCR (qPCR) was used to assess *Blattabacterium* abundance, while 16S rDNA amplicon sequencing enabled characterisation of gut microbiota diversity and taxonomic shifts across treatments. Phenotypic parameters, including developmental progression and expression of immune-related and metabolic genes, were assessed via transcriptomic profiling. Moreover, host resilience to pathogenic challenge was evaluated through survival assays following infection with Rifampicin-resistant *Pseudomonas entomophila*. This is to understand whether microbiota disruption influences host immunity. Collectively, our findings provide novel insights into the dynamic, multi-tiered interactions between host, endosymbiont, and microbiota, with implications for understanding microbial modulation of host physiology in hemimetabolous insect systems.



Microbiome structure of French Guyana *Drosophila* community

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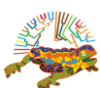
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Biological invasions threaten ecosystems worldwide, yet the multifaceted role of microbial communities in facilitating invasion success remains poorly understood. While theory suggests that microbiomes may be key players in invasion dynamics, empirical evidence from natural systems has been scarce. Leveraging the coexistence of native and invasive *Drosophila* species in the neotropics of French Guiana, we dissected their microbiome profiles across an anthropogenic gradient by revealing structuring patterns consistent with microbial acquisition and adaptation theories.

Using multi-marker amplicon sequencing (16S, ITS, EF1), we uncovered distinct microbiome signatures between invasive and native hosts, with native species harboring significantly higher microbial diversity. Intriguingly, 326 of the 327 microbial OTUs in invasive species were subsets of those found in most native hosts. While native *Drosophila* shared a core set of dominant microbes, invasive conspecifics displayed host-specific microbiome restructuring, each carving out a unique microbial niche. In natural ecosystems, invasive alien species come into contact with resident species and their associated environments, likely by acquiring the

microorganisms from local microbial communities. These interactions may contribute to the observation that the microbiome of invasive alien species often resembles a subset of the native species' microbiome. Within this context, Baas-Becking's conjecture that "everything is everywhere, but the environment selects" may help explain the differences in microbiome composition of invasive alien species upon their arrival and acclimatization. According to this framework, invasive *Drosophila* species — acting as environments for their own microbiomes — could differentially select microorganisms from resident species based on ecological or physiological affinity.

Our findings support a theoretical dynamic picture: invasion is not just about the alien invader species itself but also driven by microbiome-level negotiations. The selective acquisition and reorganization of native microbes could equip invasive species with context-dependent advantages, potentially easing their establishment. Our work supports the current shift in paradigm from host-centric invasion biology to a more holobiont perspective, where microbiome plasticity could be a hidden catalyst for invasion success.



Of mice and microbes: the gut microbiome as a driver of thermal plasticity.

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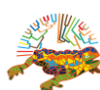
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Phenotypic plasticity enables animals to rapidly respond to changing environments, and recent advances suggest the gut microbiome could be a critical, but underexplored, avenue for such plasticity. While studies with laboratory mice (*Mus musculus*) have revealed the gut microbiome's influence on host physiology, these lack the microbial diversity and ecological context of natural populations. In wild populations, evidence linking the gut microbiome to host phenotypes is largely correlational. This leaves critical questions of how relevant laboratory insights are for wild systems, and the significance of microbiome-mediated host plasticity in nature.

To address this gap, we studied an insular population of wild house mice. We combined extensive characterisation of their gut microbiota and phenotypic variation with faecal microbial transplant experiments to test whether natural, within-population variation in the gut microbiome casually influenced phenotypic traits. In a five-year dataset (of 1665 samples from 745 individually marked mice), we first identify host and environmental drivers of gut microbiome variation. Next, using a subset of the dataset we focus on thermoregulatory

phenotypes, and ask how they are predicted by host and environmental factors, including gut microbiota composition. Finally, we conducted faecal microbial transplant experiments into germ-free mice to test whether the thermal phenotypes of wild mice are transferable with their microbiota. Our data reveal striking seasonal variation in both the microbiota and thermal phenotypes of house mice. Mice sampled in spring exhibited a 1.5°C lower body temperature than those sampled in summer and autumn. Remarkably, these seasonal differences in body temperature were transferable to gnotobiotic mice via faecal microbiota transplants. Spring microbiome transplants induced a 2.0°C lower body temperature than summer or autumn microbiome transplants. We further find a strong correlation between donor and recipient body temperature. Other traits of recipient mice also differed according to which season the microbiome donor was sampled in.

These results provide some of the first evidence that within-population (seasonal) variation in the gut microbiome mediates host physiological plasticity, underscoring its role as a critical factor shaping natural phenotypic variation and potential acclimation capacity of wild mammals.



Proteomic Profiling Reveals Brain Proteins Linked to Social Interactions in Gut Microbiota-Harbouring Honeybees

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MEETING ROOM 116, August 18, 2025, 2:00 PM - 5:00 PM

What factors regulate social behaviour has long been a key question in biology, yet its molecular determinants remain poorly understood. Social behaviour is known to be influenced by both internal physiological states and external social cues, with the gut microbiome emerging as a key internal stimulus that promotes social behaviours across various animal models. Honeybees provide a powerful model for investigating the molecular mechanisms connecting gut microbes to host social behaviours and the emergent properties of group living, as their microbiota is manipulable and their individual and group behaviours can be precisely quantified. Previous research has shown that honeybees possessing a gut microbiome exhibit more frequent and specialized social interactions compared to

gut microbiota-depleted bees. To shed light on the molecular mechanisms underlying these interactions, we performed a proteomic analysis after dissecting the brain of gnotobiotic bees into three regions: optic lobes, mushroom bodies, and antennal lobes. We found large region-specific effects of the gut microbiome on the brain proteome. We then correlated protein abundance with social behavioural data, identifying candidate proteins which may be responsible for the observed behavioural differences between microbiota-colonised and microbiota-depleted bees. Our results suggest that brain region-specific changes in protein abundance are key mediators of the interaction between the gut microbiome and honeybee social brain.



The effects of climate change on the microbial community associated with a butterfly across a 30-year period

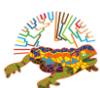
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MEETING ROOM 116, August 18, 2025, 10:30 AM - 12:30 PM

Microbial communities in host guts are essential for host fitness yet highly sensitive to environmental fluctuations such as temperature shifts. While short-term responses—like the rapid proliferation of thermotolerant bacteria after a heatwave—are well documented. However, the long-term and indirect consequences of sustained warming on these communities remain poorly understood. In this study, we analyze three decades of microbiome data from diapausing larvae of the Glanville fritillary butterfly (*Melitaea cinxia*) in the Åland Islands, Baltic Sea, to test two hypotheses: a) Sustained warming in Åland has selectively promoted or suppressed some bacteria, thereby restructuring the larval gut community over time. b) The

functional repertoire of key bacterial assemblages has evolved to help the host withstand higher temperatures—through enhanced production of protective metabolites, heat-shock proteins, or other temperature-mitigating mechanisms. To evaluate these hypotheses, we combine 16S rRNA amplicon sequencing with long-term climate record to characterize temporal changes in community structure. And use shotgun metagenomics data to reconstruct functional gene profiles of the major bacterial community. By disentangling how warming shapes not only who is present but also what they can do, this work will shed light on the resilience and adaptive capacity of host–microbiome partnerships under ongoing climate change.



The gut microbiome of invasive squirrels shows greater diversity, stability and functional potential than that of their native competitors

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MEETING ROOM 116, August 18, 2025, 2:00 PM - 5:00 PM

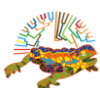
Background: Due to their influence on key host phenotypic traits and their capacity for rapid genomic and compositional shifts, highly diverse and/or plastic host-associated microbiomes may play a role in mediating animal invasions, facilitating the establishment and spread of alien species, and enhancing their competitive advantage over native species. To explore this, we analysed the gut microbial communities of invasive grey squirrels (*Sciurus carolinensis*) and outcompeted native red squirrels (*Sciurus vulgaris*) in Italy, comparing their overall composition and their plasticity in response to environmental variation.

Materials and methods: The two squirrel species were live-trapped across urbanisation gradients and seasons in multiple study areas in NW Italy, resulting in the collection of 110 and 80 faecal samples from 63 native and 45 invasive squirrel individuals, respectively. Following the standardised protocols of the Earth Hologenome Initiative (www.earthhologenome.org), we conducted shotgun metagenomic sequencing to enable genome-resolved metagenomic analyses. After assembling metagenomic reads, we reconstructed metagenome-assembled genomes (MAGs), annotated them taxonomically and functionally, and quantified their relative proportion across all samples.

Results: The metagenomic analysis resulted in 1,687 MAGs from 13 different phyla, of which 82% had no species-level annotation. The 30% of MAGs were common to both species, 29% were exclusive to native red squirrels and 41% were exclusive to invasive grey squirrels. Alpha diversity metrics were consistently higher in invasive squirrels, and the microbial communities of the two species differed significantly in structure and composition. Overall, the microbiota of native squirrels was enriched in lactic acid bacteria (fam. Bifidobacteriaceae, Streptococcaceae and Lactobacillaceae), which were absent or almost absent in invasive

individuals. The invasive host microbiota was instead enriched in Verrucomicrobiota and several Bacillota A families (e.g. Oscillospiraceae and Lachnospiraceae). While the microbiota of native squirrels were highly variable, with several samples also showing signs of dysbiosis, all invasive individuals harboured highly diverse and very consistent communities. Indeed, the relative abundance of most bacteria in native squirrels was strongly influenced by seasonality and to a lesser extent by urbanisation, resulting in significant shifts in microbiota structure over space and time. Most of these changes appear to be driven by a large increase in the abundance of lactic acid bacteria in winter and in urban areas. In contrast, the microbiota of invasive squirrels was compositionally more stable, with far fewer bacteria responding to change in any way. Despite this greater structural stability, the microbial community of invasive squirrels still showed a degree of functional variation similar to, if not greater than, that observed in native ones. The higher proportion of specialised bacteria observed in invasive squirrels may allow small changes in the abundance of a few taxa to result in large functional changes without altering community structure. Finally, dietary analysis confirmed that the two species have very different diets, with grey squirrels feeding mainly on oak acorns and red squirrels feeding mainly on conifer seeds. Indeed, the microbiome of grey squirrels was significantly enriched in tannase-producing bacteria, which are essential for degrading toxic acorn tannins.

Conclusions: The gut microbiome of invasive grey squirrels is more diverse, stable and functionally enriched than that of outcompeted native red squirrels, with a capacity for large functional variation without compromising community structure. These features suggest that microbiome-mediated plasticity may play a key role in the ecological success and competitive advantage of the invasive species.



The role of the host-associated microbiome in host tolerance and cross-tolerance to pollutants

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¹KU Leuven

MEETING ROOM 116, August 18, 2025, 2:00 PM - 5:00 PM

Populations frequently exposed to a pollutant can evolve tolerance to this pollutant. Tolerance to one pollutant can extend to other pollutants, so-called cross-tolerance. Understanding patterns of cross-tolerance is important as populations are increasingly exposed to multiple pollutants. While host mechanisms of (cross-)tolerance are well studied, and while it is known that the host's microbiome can contribute to tolerance of its host to pollutants by lowering the toxicity of pollutants for the host, it remains untested whether the host's microbiome may also play a role in driving cross-tolerance. In this study, we focused on the host-associated microbiome community as underlying mechanism of its host's tolerance and cross-tolerance.

We build on an experimental evolution trial during which *Daphnia magna* clones adapted and evolved tolerance towards the pesticide chlorpyrifos, which resulted in cross-tolerance to the pesticides malathion and carbaryl, while not affecting tolerance to the pesticide esfenvalerate and reducing tolerance to the pesticide imidacloprid and the pharmaceutical fluoxetine (as a cost of tolerance). We determined whole body microbiome community compositions (using 16S rRNA amplicon sequencing) of two chlorpyrifos-sensitive and two chlorpyrifos-tolerant clones after four-days exposures to a control or to sublethal pollutant concentrations of chlorpyrifos, malathion, carbaryl, esfenvalerate, imidacloprid and fluoxetine.

Under control conditions, chlorpyrifos-tolerant clones had a different bacterial community composition than chlorpyrifos-sensitive clones, suggesting that microbiome shifts contributed to their evolution of tolerance to chlorpyrifos. Exposure to the organophosphates chlorpyrifos and malathion did not change the bacterial community composition of the chlorpyrifos-tolerant clones compared to the control, whereas chlorpyrifos-sensitive clones did show compositional changes. This suggests that the microbiome of chlorpyrifos-tolerant clones is adapted to organophosphates and includes bacteria (e.g.

Pseudomonas, *Enterobacter*) which may possess organophosphate-degrading enzymes. These bacteria may contribute to the host's tolerance to chlorpyrifos and cross-tolerance to malathion.

Although there is cross-tolerance among chlorpyrifos and the carbamate carbaryl at the host level, this was not observed at the microbiome level as exposure of the chlorpyrifos-tolerant clones to carbaryl shifted their bacterial community composition towards that of the sensitive clones exposed to carbaryl. This suggests that bacteria mediating tolerance to chlorpyrifos cannot degrade carbaryl (because of their different molecular structure). Also for imidacloprid, the bacterial community composition of chlorpyrifos-tolerant clones shifted towards the composition of chlorpyrifos-sensitive clones upon exposure, indicating no cross-tolerance at the microbiome level. For esfenvalerate and fluoxetine, no microbiome changes were observed in either tolerance type.

Our results show that host-associated microbiome responses to pollutants are pollutant-specific and depend on host tolerance type, consistent with the idea that evolution of tolerance in the host is associated with changes towards a microbiome that is capable to degrade specific types of pollutants. The evolution of chlorpyrifos tolerance in *Daphnia* was associated with microbiome shifts contributing to both tolerance to chlorpyrifos and cross-tolerance to malathion. However, this microbiome-mediated tolerance was not observed for carbaryl, highlighting that microbial and host mechanisms of cross-tolerance are complementary. Microbiome-mediated cross-tolerance appears to depend on the pollutant's chemical structures, while host-level cross-tolerance is related to pollutant's shared modes of action and detoxification pathways. This study provides the first experimental indication that host-associated microbiomes influence not only the host's tolerance but also its cross-tolerance to pollutants, underscoring the microbiome's potential in host adaptation to pollutants





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**CONGRESS OF THE EUROPEAN SOCIETY FOR
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S08 - Cooperation, Conflict and the Evolution of Socially Transferred Materials



Evolutionary Adaptation to Juvenile Malnutrition and Investment in Sexual Conflict

Canal Domenech B¹, Kawecki T¹

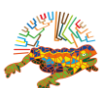
¹University Of Lausanne

POSTER SESSION 3 - P03.157, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

In many species, including *Drosophila*, males allocate resources to costly sexual traits that mediate competition for female gametes and often result in sexual conflict. Under chronic nutrient limitation, selection may be predicted to favor reduced investment in these traits because their energy and resource costs are likely to have a greater impact on survival of nutritionally stressed individuals. On the other hand, exposure to nutrient shortage over multiple generations should promote physiological adaptation to nutrient shortage, leading to a more efficient resource use, which would leave more resources available to be invested in sexual traits.

We test these contrasting predictions about how long-term adaptation to malnutrition influences the evolution of male

reproductive traits, using experimental evolution in *Drosophila melanogaster*. We compare 'selected' males from replicate populations that evolved on a nutrient-poor larval diet for over 300 generations to 'control' males evolved on a standard diet, testing each on both poor and standard diets, which allows us to study both the evolutionary responses and phenotypic plasticity. We focus on traits mediating postcopulatory components of sexual selection and conflict, including success in sperm competition and the ability to inhibit female remating, as well as the underlying investment in seminal fluid proteins, a key factor in male competitive reproductive success. Our results throw light on how an interaction between nutritional conditions and metabolic adaptation shapes sexual conflict.



Fecundity help may be more common than survival help in realistic ecological scenarios

Bolton M¹, Early R¹, Kuijper B¹

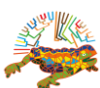
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POSTER SESSION 3 - **P03.150**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The evolution of social behaviours - such as altruism - strongly depends on an organism's lifecycle. Existing theoretical predictions on the evolution of altruism typically consider life histories in which population turnover is driven by adult mortality in response to external factors (death-birth), or life histories in which births drive population turnover by triggering adult deaths (birth-death). The first of these - death-birth- is a simplification of species lifecycles in which adults must die before juveniles have a chance to establish as breeders. Conversely, birth-death can be thought of as representing species where juveniles put survival pressure on adults. Which mechanism is chosen has profound consequences for the evolution of help.

However, death-birth and birth-death sit at two extremes. Therefore, we aim to represent a more natural scenario by developing two hybrid update mechanisms. These occupy a middle ground in which

population turnover is driven both by external effects on mortality, and intergenerational competition for survival triggered by the birth of juveniles. We use two slightly different mechanisms; one in which all adults and juveniles compete for survival in the same pool (full scramble), and the second in which individual adults compete independently against subsets of juveniles (adult defence). In both, altruism is only able to evolve when help affects fecundity, not when it affects survival. From this finding, we suggest natural populations may be more likely to exhibit fecundity-based help. Survival-based altruism may only arise in the presence of additional population characteristics which increase the evolutionary scope for help. Additionally, because generational overlap promotes the evolution of help, we suggest that survival-based feedback loops may arise when help affects the rate of population turnover.



Reciprocal influence on viral load and effects on virulence in co-infections with latent ALSV and phylogenetically distinct viruses

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POSTER SESSION 3 - **P03.179**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Plant virus co-infections are ubiquitous and can dramatically alter disease outcomes. Understanding the interactions between different plant viruses is crucial for managing viral diseases and ensuring global food security. This study focuses on Apple latent spherical virus (ALSV), a bipartite virus known for its broad, asymptomatic host range, and its influence on a diverse set of co-infecting viruses. Utilizing *Nicotiana benthamiana* as a model host, we investigate the impact of ALSV co-infection on viruses belonging to the Bromoviridae, Virgaviridae, Alphaflexiviridae, Potyviridae, Secoviridae and Caulimoviridae families.

We hypothesize that ALSV, despite its latent nature, may significantly influence the dynamics of co-infecting viruses and vice versa. To test this, we quantified individual viral loads in both mono- and co-infection scenarios, focusing on two key aspects:

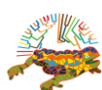
1. Impact on Viral Accumulation: Does the presence of ALSV enhance, reduce, or have no effect on the accumulation of other viruses?
2. Genomic Formula Stability in Multipartite Viruses: any of the viruses in this study,

including ALSV, have multipartite genomes. Does co-infection alter the RNA ratios of these viruses, potentially impacting their replication and functionality?

Furthermore, we assessed virulence through plant growth measurements to determine the nature of the virus-virus interactions (neutral, antagonistic, synergistic). By exploring these facets of co-infection, we aim to shed light on the complex interplay between ALSV and a diverse set of plant viruses, and ultimately determine if phylogenetic relationships influence interaction outcomes.

Our results revealed a range of interactions, including neutral, neutral-antagonistic, on viral load and synergistic effects for virulence in a particular case. Interestingly, the genomic formula of some multipartite viruses may be modulated in co-infection situations. These findings highlight the complex nature of plant-virus interactions and emphasize the importance of understanding the implications of co-infections for plant health and disease management.

Keywords: plant viruses, co-infection, viral load, genomic organization, virulence



Sexual Selection and the Evolution of the Mammalian Brain and Social Cognition: A Proposal on the Evolution of Selfishness and the ‘Male Brain Meme’ Concept

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POSTER SESSION 3 - P03.159, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Our research group recently reported that selfish DNA contributes to the emergence of a novel sex-determining gene and to biased subgenome evolution following interspecific hybridization (1, 2). In the present study, I shift focus from the molecular level to the individual and population levels to explore the evolution of selfishness in mammals and humans.

Sexual size dimorphism in vertebrates varies widely across taxa. In amphibians, females are typically larger, whereas in fishes, reptiles, and birds, the patterns depend on ecological and phylogenetic contexts. However, in most mammals, males tend to be larger—a trend that can be explained by Darwin’s theory of sexual selection, particularly male–male competition. This presentation offers a novel perspective by examining the human social brain through the lens of evolutionary biology, focusing on mammalian evolution, brain sexual dimorphism, sex steroid hormones, social behavior, and the evolution of human society.

Nocturnality and Male–Male Competition: Mammalian ancestors are believed to have been nocturnal. This section explores the evolutionary linkage between nocturnality, visual gene adaptation, and sexual selection, and proposes the concept of the “mammalian Male Brain,” characterized by

increased aggression and combativeness. Harem structures exemplify polygyny driven by such traits. The mammalian male brain can thus be viewed as an expression of selfishness at the individual level.

Sex Steroid Hormones and the ‘Male Brain’: In most mammals, androgens shape the structure of the male brain during fetal development. I introduce the concept of the “Residual Male Brain,” referring to the persistence of aggression and violence even after androgen levels decline in adulthood.

Cooperative Aggression and Group-Level Selfishness: I analyze violent intergroup aggression in male groups of chimpanzees and humans, proposing that intragroup cooperation against outgroup males is a foundational mechanism of war. This cooperative aggression can be interpreted as a form of selfishness at the group level.

Proposal of the ‘Male Brain Meme’: I define the “Male Brain Meme” as a cultural construct based on evolutionarily derived male aggression. It supports dominance structures characterized by authority and power. I discuss its evolutionary implications and its role in sustaining male-dominated societies and warfare.

1. Hayashi et al. *Mol Biol Evol* 39: msac138, 2022.
2. Suda et al. *Genome Biol Evol* 16: evae179, 2024.



Studying the evolutionary impact of parental influence on mate choice in human societies

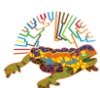
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POSTER SESSION 3 - P03.155, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Human parents exert significant influence over their children's choice of mating partners in many modern societies, reflecting a trend likely prevalent throughout our evolutionary history. This influence creates an evolutionary conflict between parents and their adult offspring, whose fitness interests may diverge regarding the latter's choice of partner. This is especially true if parents can continue to produce offspring directly, rather than investing in their (less genetically related) grandoffspring. Consequently, parents may

be expected to prioritise factors such as resource acquisition when choosing partners for their children, often mediated through practices like bride-prices and dowries. However, this directly conflicts with the offspring's aim to maximise their own fitness through partner selection. Through theoretical analysis and agent-based simulations, we explore this conflict's evolutionary implications, including its effects on mate-choice strategies and demographic trends.



Substrate hardness and social environment drive egg clustering behaviour in *Drosophila melanogaster*

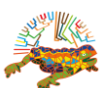
Fowler E¹, Friend L¹, Churchill E², Yu D¹, Archetti M³, Bourke A¹, Bretman A³, Chapman T¹

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POSTER SESSION 3 - P03.140, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Egg placement decisions can have profound fitness consequences. One oviposition strategy reported across taxa is laying eggs in clusters, including with eggs of conspecific individuals. However, the drivers of egg clustering behaviour remain poorly understood. Egg clustering could have significant fitness benefits, including the potential to share resources provided by a neighbouring egg (e.g. externally deposited microbes or defensive compounds). Additionally, offspring emerging from a cluster of eggs may cooperate or compete over access to nutrients and other resources. Using the fruitfly, *Drosophila melanogaster*, we tested: 1) whether females plastically adjust egg clustering based on substrate hardness; 2) whether social context influences egg placement on hard and soft substrates; and 3) which sensory inputs underlie egg clustering behaviour.

Females laid fewer eggs, but increased egg clustering, on harder substrates. Grouped females laid more eggs than isolated ones, but this difference was only found for hard substrates. Surgical manipulations of key sensory organs revealed that the antennae and labellum were essential for egg laying on hard substrates only, while females lacking foretarsi laid eggs on all substrates but no longer exhibited egg clustering plasticity. The results revealed that egg clustering behaviour is plastic according to substrate condition, and that social cues interact with other environmental factors to influence oviposition decisions. Mechanosensory inputs via the tarsi are likely important for females to fine-tune egg positioning. This characterisation of egg clustering behaviour in *D. melanogaster* enables future investigation of the fitness consequences of fine-scale oviposition strategies.



The cooperation inside us: investigating bacterial cooperation in the human gut

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POSTER SESSION 3 - P03.162, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The bacteria in our gut are thought to play a major role in our development, behaviour and disease. Rather than acting in isolation, bacteria are extremely social. Cells secrete molecules which benefit nearby cells, with this cooperation allowing the invasion of hosts and acquisition of nutrients. However, the evolution of cooperation is not well understood in our gut communities. Particularly, it is unclear what kind of selection pressures influence cooperation in gut bacterial communities. To explore this, we analysed gut metagenome data from 231 humans. We calculated the species' relatedness (how similar strains are to one another), polymorphism and protein divergence of genes encoding for cooperative and private traits. We found that the number of cooperative genes varied greatly between species, but the proportion

of cooperative genes increases as species' relatedness increases. We then examined whether genetic diversity in these genes was correlated with the relatedness of species. Evolutionary theory suggests that genes for cooperation should be under relaxed selection, which should mean that diversity in genes for cooperative relative to private traits would increase as relatedness decreases. However, opposite to these predictions, we found that species with higher relatedness showed higher relative polymorphism in genes for cooperative compared to private traits. This suggests that other factors are at play. For example, selection on cooperation in the gut might be different compared to other systems studied so far. Further investigation and statistical analysis are under way to find out.



The socio-sexual environment influences diet and oviposition site choice in female fruit flies

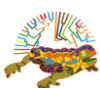
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POSTER SESSION 3 - **P03.146**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The balance of macronutrients that animals consume has key impacts on reproduction and lifespan, and individuals are able to actively regulate their macronutrient intake by feeding from diets of differing composition. The social and sexual environment can also be important drivers of diet choice behaviour. For example, male harassment pushes females away from high-quality foraging patches in some insects, while foraging in groups can result in collective feeding from a single patch. However, it is unknown how female insects balance a need to optimise macronutrient intake against cues of patch quality provided by conspecifics of the same and opposite sex and different risks of male harassment across food patches. In this study, we investigated female feeding choice in response to same-sex and opposite-sex social environments. Female feeding choice between two diet patches differing in macronutrient composition were measured in fruit flies (*Drosophila melanogaster*) in experimentally controlled socio-sexual

groups. We experimentally manipulated whether females could physically interact with non-focal individuals or could only perceive cues of non-focal individuals that were restricted behind barriers. Contrary to our predictions, females did not exhibit a preference for protein-rich diet patches, and macronutrient preference was not modified by harassment from unrestricted males. Interestingly, females did not avoid restricted males, despite a risk of harassment. Instead, females moved to the most populous patch, regardless of macronutrient content. These results suggest that females might have used the presence of males as a signal of foraging patch quality and prioritised social information above both direct information on nutrient quality and harassment risk. I will also discuss patterns of female feeding and oviposition when patches contain social cues of male versus female conspecifics, to further dissect the role of sex in social signals of foraging quality.



Ejaculate-driven trade-offs: increased fertilization success comes at the cost of lower offspring quality

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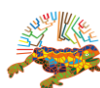
MEETING ROOM 131, August 21, 2025, 10:30 AM - 12:30 PM

Besides DNA, fathers can transfer non-genetic factors during copulation, which can affect offspring phenotype (i.e., ejaculate-mediated paternal effects; EMPEs). Today it remains unclear if EMPEs are adaptive, a selectively neutral side effect of copulatory interactions, or even detrimental for the offspring. The latter scenario could arise if ejaculate components that increase male fitness, for example through increased fertilisation success, have detrimental effects on individual offspring quality (quality – quantity trade-off), resulting in parent-offspring conflict. EMPEs have been studied mostly in invertebrate species, and their generalisation—or even their presence—in other taxa is poorly known.

Male quail of the genus *Coturnix* are unique among birds in that they produce a white foam, secreted by the proctodeal (or cloacal) gland independently of sperm and seminal fluid, which is transferred during copulation. This foam consists of viscous, aerated glycomucoproteins, which boosts fertilisation success and may influence sperm competition outcomes. Certain foam components have been shown to fuel sperm, enhancing its motility, velocity and longevity, with glucose and lactate identified as main

energy suppliers. However, the factors that shape heterogeneity in foam composition among males and the consequences of foam transfer for the offspring remain unknown. Here we tested whether foam components that improve male fertilisation success influence offspring quality at birth in Japanese quail (*Coturnix japonica*). To this end, we took repeated foam samples from males to quantify foam mass and concentrations of foam lactate and glucose and to assess trait repeatability. Then, males were mated with a randomly allocated female, and we quantified offspring body mass and blood telomere length (by TRF), a biomarker of physiological damage and ageing.

We found a high repeatability of all foam traits. While no association between foam components and offspring body mass was found, foam glucose concentrations were negatively associated with offspring telomere length at birth ($r = 0.58$). These findings highlight that non-genetic foam components transmitted during copulation affect offspring phenotype and that fathers and offspring are in conflict over copulatory foam composition in this precocial bird.



Larval Altruism and the Development of a Worker Caste - Theoretical Investigations into Primitively Eusocial Wasp Larvae as Altruists.

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MEETING ROOM 131, August 21, 2025, 10:30 AM - 12:30 PM

For decades, both empirical and theoretical research on the eusocial insects has focussed on the role of adult altruism, but the possible importance of larvae as helpers has received surprisingly little attention. Larvae of social wasps (and some other Hymenoptera) act as a ‘communal stomach’, digesting solid food provided by workers and regurgitating it to adults (‘larval-adult trophallaxis’) in a nutritionally rich ‘saliva’. This act of donation is potentially costly to larvae, hindering their future reproductive prospects. In 1916, Émile Roubaud hypothesised that larval–adult trophallaxis is the principal attractant of adults to the nest, placing larval actions at the foundation of eusociality. Despite this extraordinary claim, larval–adult trophallaxis has received little empirical attention and, since the inception of kin selection theory, no theoretical attention. Using kin selection

methodology and agent-based simulation, we show that the development of an adult worker caste in primitively eusocial insects can occur during larval development. Small differences in larval development (e.g., in provisioning by adults or digestive ability) can drive large differences in larval and adult strategies. By modelling ‘larval altruism’ games in which larvae exhibiting higher levels of altruism (donation of resources to adults by trophallaxis) hinder their own reproductive prospects in adult life, we find a ‘larval altruist caste’ can develop prior to adult workers and that larval altruists are subsequently more likely to be adult altruists. Our results highlight the importance of larvae in social insects, and provide testable predictions for empirical research into the long-neglected role of larvae in the evolution of eusociality.



Maternally secreted protein ensures symbiont transmission in tortoise beetles

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MEETING ROOM 131, August 21, 2025, 10:30 AM - 12:30 PM

Symbiont transmission ensures the maintenance of mutualisms. Insects frequently rely on socially transferred materials to endow their offspring with beneficial microbes. Here, we characterized how tortoise beetles (Coleoptera: Chrysomelidae: Cassidinae) vertically transmit their obligate digestive symbiont, *Stammera capleta*. Tortoise beetles deposit symbiont-bearing caplets at the anterior pole of each egg during oviposition. These caplets contain spherical secretions that embed *Stammera* extracellularly and are consumed by larvae prior to hatching to initiate infection. As *Stammera* subsists outside the host for up to 11 days during transfer, a protective mechanism is essential for its survival. We discovered that these spherical secretions are primarily surrounded by a dense, fibril-like matrix composed of a host-derived protein. This protein belongs to the Yellow family and is uniquely and highly expressed in female ovary-associated glands. Functional knockdown of this protein

through RNA interference results in degenerated spheres. *Stammera* is more susceptible to desiccation in the absence of the host-derived Yellow, underscoring the critical role that this protein plays to ensure extracellular symbiont transmission in tortoise beetles. Additionally, we identified a novel organ specialized for storing these Yellow-surrounded spheres prior to egg deposition. Phylogenetic and structural analyses reveal that Yellow is shared across multiple tortoise beetle species, suggesting an evolutionarily conserved strategy for symbiont protection and transmission. Despite their diverse roles in pigmentation, cuticle formation, and nutrition in insects, Yellow proteins have not been previously demonstrated to play a role in symbiont maintenance and propagation. Our findings demonstrate the evolutionary co-option of a Yellow protein for extracellular symbiont protection, highlighting how host traits evolve to mitigate the challenges of symbiont transmission and stability.



Metabolic Division of Labour as an Evolutionary Ratchet for Social Systems

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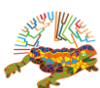
MEETING ROOM 131, August 21, 2025, 10:30 AM - 12:30 PM

Behaviours that transmit metabolised materials from one individual to another allow the genome of one individual to influence the fitness of another. This decoupling of resource acquisition, processing, and use across individuals results in metabolic division of labour, a fundamental process underlying diverse forms of biological cooperation. I propose that metabolic division of labour through private channels of exchange serves as an evolutionary ratchet driving increasing interdependence across multiple levels of biological organisation.

Across biological systems, we observe similar patterns where routes of metabolic exchange create dependencies: parents provide nutritive secretions to offspring, endosymbionts supplement host metabolism, and mutualistic partners exchange vital nutrients. While these relationships initially evolve due to mutual benefits, the establishment of private channels for metabolic exchange initiates a

ratchet-like process of increasing commitment. As partners invest in specialised structures for metabolic transfer, they become increasingly interdependent – losing metabolic capabilities and developing dependencies. Private channels have clear risks and are often avoided, despite efficiency costs. I will discuss three main systems including endosymbioses, social insects, and vertebrate parental care.

By viewing the evolution of cooperation through the lens of socially transferred materials and their resulting metabolic interdependencies, we gain insight into both the innovations that enable cooperation and the constraints that maintain it. This perspective helps explain not only how cooperative relationships become entrenched but also why they often intensify and sometimes become fragile over evolutionary time, as these dependencies shape the ecological and evolutionary landscape.



Predictions of sexually antagonistic co-evolution extend to the molecular level of seminal fluid and female reproductive tract proteins.

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MEETING ROOM 131, August 21, 2025, 10:30 AM - 12:30 PM

Background: Sexually antagonistic co-evolution (SAC) is a potent driver of evolutionary dynamics, with males and females evolving traits that enhance their own fitness but harming the opposite sex. Theory predicts that levels of exaggeration at resistance and persistence traits should predict the outcomes of mating interactions and which sex has an advantage in sexual conflicts. SAC is well-studied phenotypically and these predictions have been confirmed. However, SAC, and the effects of molecular “exaggerations”, remain poorly understood at the genetic level. This is because the genes underlying the best examples of SAC are not well-known.

One exception is the iconic sex-peptide (SP) network in *Drosophila melanogaster*. The SP-network comprises a set of socially transferred materials - male seminal fluid proteins - that interact with proteins expressed in the female reproductive tract. These interactions mediate sexual conflicts over female re-mating rates and ovulation. Males benefit from reducing female re-mating rates and increasing ovulation to ensure and increase paternity, while females should prefer to re-mate when advantageous to do so and delay ovulation to exercise choice. These conflicts could lead to SAC between male- and female- components of the SP-network. However, the role of natural genetic (co-)variation in the SP-network in shaping these behaviours is understudied.

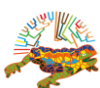
Materials and methods: We capitalise on this system and test the predictions of SAC at the molecular level using population genomic and isofemale line resources from wild populations of *D. melanogaster*. We first use population genomic analyses to evaluate patterns of co-variation in molecular evolution of the male- and female- components of the SP-network

across *D. melanogaster*. We identify populations where the relative degree of molecular “exaggeration” (genetic differentiation) implies an advantage in sexual conflicts to males, females, or neither sex. We then conduct (re-)mating experiments in the lab, including follow-up fitness assays, using flies collected from 6 European populations.

The hypothesis that these genes co-evolve by SAC predicts that; a) there should be co-variation in population genetic differentiation between male- and female- components of the SP-network, and b) that populations with an inferred female advantage will show higher re-mating rates and increased direct benefits (higher offspring production and reduced fitness costs) to females compared to populations with inferred male advantage.

Results: We find strong support for both of our main predictions. We find strong support for co-variation, indicating co-evolution between the loci. Populations with an inferred female advantage show higher rates of re-mating than populations with an inferred male advantage. Moreover, female-advantage populations also have a longer time until the peak of offspring production, reflecting a slower rate of ovulation. This pattern suggests that females may be more able to resist also the ovulation inducing effects of SP.

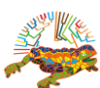
Conclusions: Our study highlights the importance of considering co-evolution between donors and recipients of socially transferred materials. We show that patterns of molecular co-evolution between the transferred male seminal fluid proteins and proteins of the female reproductive tract predict outcomes of mating interactions. Our findings support a role for SAC in the evolution of these molecules and, for the first time, extend SAC theory to the molecular level.





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S09 - Craniofacial Evolution in Vertebrates



A matched genome-phenome dataset of the world's wolves (*Canis lupus*) and dogs (*Canis familiaris*)

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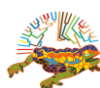
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POSTER SESSION 3 - P03.130, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

A common feature of domesticated animals is a shortening and widening of the skull, along with reduced brain size, tooth size and body mass. Fortunately, skulls tend to be well preserved in large numbers in natural history collections, documenting variation in wild species and their domestic relatives over time and space. When combined with genomic data, this material presents a valuable opportunity to reconstruct the timing and sequence of events during domestication, including genetic, morphological and behavioural changes. Here, we present a matched genome-phenome library of wolves (*Canis lupus*) and dogs (*Canis familiaris*) spanning ~350 individuals, including >100 dog breeds and >200 wolves collected across 19 countries. MicroCT scanning was used to generate high-resolution 3D skull models, paired with whole genome sequencing for each individual. We exploit this database to

explore canid skull morphology and its genomic basis using automated landmarking tools, to assess how domestication influences fine-scale shape variation as well as skull asymmetry, modularity and integration. We use GWAS to identify key genomic regions associated with skull evolution, and to interrogate loci known to influence skull shape in dog breeds. Integration of these unique, yet complementary datasets will allow us to investigate complex evolutionary processes, and to pinpoint signatures of selection in skull morphology associated with domestication. Our approach provides a new template for the integration of genomic and morphological data in the study of evolutionary processes, particularly for when genetic and morphological data disagree.



Applying ancient DNA extraction methods to improve the recovery of genomic material from modern bone samples

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POSTER SESSION 3 - **P03.110**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Gene regulation plays a central role in shaping the developmental and evolutionary morphology of the skull. DNA methylation has emerged as a powerful proxy for inferring gene regulatory activity across tissues. However, our understanding of intra- and inter-individual variation in gene regulation within the craniofacial skeleton remains limited. This gap is partly due to the rarity of opportunistically available skeletal tissues from deceased individuals and the inherently low cellular density of bone, which restricts the recovery of genomic material. As a result, extracting DNA from small craniofacial bone samples – even those from well-preserved modern specimens – remains technically challenging, requiring careful optimization to maximize yield and quality. Here, we conducted a pilot study to assess whether ancient DNA extraction methods can be successfully applied to modern craniofacial bone samples to recover genomic material.

We obtained a wild boar skull with a known postmortem interval and a pig skull sourced from the food industry, and froze these specimens at -80°C. Bone cores (N = 8) were extracted from various craniofacial regions using a 50 mm diameter drill. Cortical bone was separated from trabecular bone with sterile scalpels, and any remaining soft tissue was removed by washing the cores in PBS. Bone powder was then prepared from each cortical bone sample using a freezer mill. We applied and compared two DNA extraction protocols, both designed to maximize DNA recovery: a manual phenol-chloroform method previously used on ancient and modern bone samples (Method 1) and a silica-based method optimized for ancient bone (Method 2). DNA yield and quality were assessed using a Qubit fluorometer and an Agilent TapeStation, respectively.

When accounting for the initial amount of bone powder, Method 2 consistently yielded significantly more DNA than Method 1 (average total amounts: 2535 ng versus 318 ng, respectively; p-value: 0.004). Moreover, Method 1 was less reliable and sometimes failed to recover any DNA (3 out of 8 samples). Despite lower yields, Method 1 exclusively recovered long DNA fragments (average size: 30616 bp), as opposed to Method 2 which recovered both long and short DNA fragments (range: 590-25447 bp). These fragment size distribution differences are further reflected in the resulting DNA Integrity Numbers (DIN), with the average score of 7.9 for Method 1 being higher than the average score of 4.9 for Method 2. Although Method 2 retained many small DNA fragments, large fractions of the total DNA also contained long fragments (average size: 21169 bp) which have comparable lengths to those recovered in Method 1 (p-value: 0.8).

Applying Method 2 resulted in significantly higher DNA yield than Method 1, but also recovered a broader distribution of fragment sizes. This aligns with our expectations, as both methods were originally developed for ancient DNA recovery, but Method 2 has been specifically optimized to capture highly degraded DNA with small fragment sizes. Overall, our primary goal is to maximize DNA yield from small bone samples, while not substantially sacrificing on DNA quality. We show that Method 2 enables consistent recovery of sufficient DNA yield and quality for downstream applications. Moving forward, we plan to expand our testing of Method 2 to craniofacial skeletal tissues from a broader phylogenetic distribution of species with different degrees of preservation to investigate variation in skeletal DNA methylation patterns.



Biomechanical adaptation to an anthropogenic diet in a human-commensal bird

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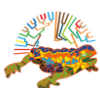
POSTER SESSION 3 - **P03.115**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The vertebrate craniofacial system plays a pivotal role in many ecological contexts including defence, mate acquisition, and feeding. Performance traits, such as bite force, provide valuable insights into the relationship between variation in craniofacial morphology and the ecological niche of populations. The anthropogenic niche is characterised by human-induced modifications in resource availability, for example the introduction of novel foods or increased abundance of human foods. Populations which depend upon these human resources, known as human-commensals, therefore provide a fascinating opportunity to consider craniofacial evolution within the context of dietary adaptation to human-modified environments. The house sparrow (*Passer domesticus*) is a globally distributed human-commensal species, found in human-modified environments across the world. The species' association with humans arose during the Neolithic, when human societies transitioned from hunter-gatherer to agricultural-based lifestyles. This increased human association likely led to a change in feeding ecology, specifically an increased reliance on tough, domesticated cereal grains such as wheat. Whilst previous genomic analyses have identified signatures of selection on genes associated with

craniofacial morphology in human-commensal populations, the phenotypic implications of this remain unexplored.

We determined whether a transition to a hard, anthropogenic seed diet is associated with adaptive changes in the structure and function of the feeding apparatus in house sparrows. Firstly, we used a combination of faecal metabarcoding and stable isotope approaches to characterise the diet of human-commensal and non-commensal sparrow populations. We then related dietary differences between populations to measurements of bite force, skull and beak morphology, and jaw muscle architecture.

As predicted, we observed stronger bite forces in human-commensal populations, associated with important changes in craniofacial morphology, including increased head width and beak depth. Furthermore, we identified differences in underlying jaw muscle architecture, including increased jaw muscle mass in human-commensal populations. Taken together, our findings show dietary transitions are associated with morphological and biomechanical adaptation, highlighting the importance of craniofacial evolution in the ability to thrive in human-modified environments.



Ecomorphological diversification of Caviomorph rodents and the role of environmental change as a driving force

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POSTER SESSION 3 - **P03.129**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

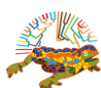
Adaptive radiations are regarded as a major driver of biological diversity and ecomorphological disparity. These radiations are initiated by ecological opportunity, where intrinsic innovation can be exploited to occupy new niches. The generation of new available niches might depend on external factors, such as environmental changes or the colonization of new geographical regions. Caviomorpha, an infraorder of Neotropical rodents, is characterized by both its high species richness and its eco-morphological disparity, and is considered an example of an adaptive radiation. This clade diversified in South America during a period of profound environmental change. Although events such as the Eocene-Oligocene transition cooling and the uplift of the Andes have been proposed as potential drivers of caviomorph diversification, direct assessments of their role on the evolution of phenotypic diversity remain limited. Additionally, during this diversification process, Carpomyinae, a clade within Caviomorpha, arrived and diversified in the Caribbean Islands. Despite this, a comparison of shape evolution between continental groups and those which later diversified in the islands has not been investigated. This clade provides an opportunity to not only study the effects of environmental change in phenotypic diversification, but also to compare the effects of these in both continental and insular settings.

To address these knowledge gaps, we compare phenotypic diversification in Caviomorpha with temporal changes in environmental variables. We assessed cranial and mandibular shape diversity for 170 species of caviomorph rodents (68% of the total diversity) using landmark-free 3D geometric morphometrics. Appendicular morphology was analysed through the measurement of continuous linear traits in 58 species. Principal

component analyses (PCA) were generated from these data to examine the phenotypic diversification over time, using phylogenetic comparative methods to investigate trait evolution. This also included the fitting of different evolutionary models, including a Brownian Motion model, an Early Burst model, and two different Ornstein–Uhlenbeck models where the long-term mean is dependent on two key environmental variables: temperature and Andean elevation rates.

Preliminary analyses show a strong phylogenetic signal and a clear separation of the families in the morphospace. Echimyidae, the most species-rich family, shows a clear separation in postcranial shape between continental clades and the insular clade Capromyinae, which contrasts with previous studies on mandibular morphology. Ctenomyidae, the second most species-rich family, shows a high morphological diversity compared to other groups, despite its seemingly ecological homogeneity. Model fitting also allows for understanding of how external variables may drive phenotypic diversification in this key mammalian clade and contribute to creating new ecological opportunities.

This study provides deeper insights into the relationships between the environment and the origins of ecological diversity, particularly in the context of major environmental transitions and the diversification events generated by them. The study also provides a comparison of the diversification process between the mainland and in island environments, and how different geographical contexts shape phenotypic evolution. It further underscores the importance of examining both internal and external drivers in adaptive radiations and highlights how these radiations represent complex interactions between the environment and the species involved.



Evidence for tonotopic organization in zebrafish otolith organ: Implications for the evolution of vertebrate auditory systems

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POSTER SESSION 3 - P03.133, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

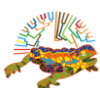
Background: Auditory information is vital for many animals. Frequency discrimination of sound is fundamental to auditory perception. In terrestrial vertebrates, different frequencies of sound are encoded and processed by hair cells (HCs) in the auditory sensory organs (e.g., cochlea in mammals) and brain neurons that are spatially orderly arranged, forming tonotopic maps. How this frequency-selective response regionalization emerged in the process of diversification of vertebrate species remains an open question. Since both the auditory and vestibular systems are thought to have evolved from a common ancestral sensory system, comparative analysis of acoustic-vestibular systems across species offers key insights into this evolutionary process. The otolith organs in the inner ear primarily function as linear acceleration detectors. These organs contain a specialized region called the striola, which is characterized by distinct morphological and physiological properties of HCs. The striolar HCs transduce phasic stimuli, allowing them to detect rapid changes in head acceleration. Fish hear sound using their otolith organs, although they do not have the cochlea. Although frequency-selective regionalization in fish otolith organs has been indirectly suggested, neural activity patterns during various frequencies of the stimulus remain unknown.

Materials and methods: To address how otolith organs respond to various frequencies of vibration, we developed microscope and stimulus apparatuses to image neural activity during vibratory stimulus. This stimulus mimics head motion and the particle motion

component of sound in water. We used larval zebrafish as a model organism to image HC activity in vivo (Tanimoto et al., Nat. Commun., 2022). We applied sinusoidal acceleration in a single axis with frequencies ranging 1 and 1,000 Hz, which enabled systematic characterization of the frequency response.

Results: In vivo Ca²⁺ imaging revealed spatially segregated, frequency-dependent responses in HCs in the utricular otolith organ of zebrafish larvae. Low frequency vibration (<20 Hz) activated most HCs that have hair bundle polarity parallel to the stimulus axis except a small number of HCs in the striola. As the vibration frequency increased, responsive HCs became confined to the striola region. Higher frequency vibration activated only a small population of HCs in the striola that did not respond to the low frequency stimulus, demonstrating a clear tonotopic organization.

Conclusions: These results demonstrate the presence of tonotopic organization in the fish otolith organ. Given the high conservation of vestibular organ structure and function across vertebrate species, our findings suggest that tonotopy represents an ancestral feature acquired in the common vertebrate ancestor, although the independent acquisition of tonotopy in fish through convergent evolution cannot be completely ruled out. This study provides important evolutionary insights into the acquisition of acoustic frequency discrimination in vertebrate auditory systems.



Evolutionary dynamics of gene and isoform regulation underlying jaw diversification in parallel cichlid adaptive radiations

Singh P¹, Ahi E², Duenser A³, Seehausen O¹, Sturmbauer C⁴

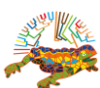
¹University Of Bern, ²University of Helsinki, ³Institute for Freshwater Ecology and Fisheries Management,

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POSTER SESSION 3 - **P03.098**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Cichlid fishes exhibit extraordinary craniofacial diversity that allowed that to rapidly adapt to novel niches as ecological opportunity arose. While it is generally accepted that variation in gene expression (GE) contributed to this process, it is less understood how alternative splicing (AS), a major source of transcriptional variation, shaped this diversity. Here we investigate the landscape of AS and GE in the oral and pharyngeal jaws of three iconic adaptive radiations of African cichlid fishes that evolved divergent and convergent trophic adaptations within <16,000 to 3.8 million years. We show that jaws of ecologically divergent species from younger radiations exhibit greater differences in splicing than those from the older radiation. Most of these differentially spliced isoforms that shaped diverse jaws arose from ancestral standing variation, which was also expressed at low levels in non-radiating species, and increased in frequency during the adaptive radiation process. We identified several

novel isoforms associated with cartilage development that emerged within each lake radiation and were differentially incorporated in the jaws of herbivorous vs. carnivorous species. While hundreds of genes were differentially expressed and spliced between divergent jaws, only a handful of genes were repeatedly used to give rise to similar jaw shapes in three cichlid radiations. Furthermore, we found that the parallel modular expression of thousands of genes may underlie the differences between the functionally decoupled oral and pharyngeal jaws across all three cichlid radiations. Of note were genes from the *alx* craniofacial skeletogenesis transcription factor family (*alx1*, *alx4*), of which *alx1* is associated with beak divergence in Darwin's finches. We suggest that a complex interplay of GE and AS underlies jaw diversification cichlid adaptive radiations, with differences in splicing arising from ancestral variation enabling rapid ecological diversification during speciation.



Exploring the genetic correlations of craniodental size dimensions in a pedigreed baboon colony

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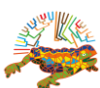
⁵National Park Service

POSTER SESSION 3 - P03.106, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Phenotypic correlations between cranial and dental traits have long been central to primate paleontology and osteology. While quantitative genetic approaches have clarified the genetic basis of variation in either cranial or dental traits independently, few studies have investigated both anatomical regions simultaneously. In this preliminary study, we assess genetic correlations among craniodental traits in a captive baboon pedigree from the Southwest National Primate Research Center (Texas, US). We analyzed linear measurements reflecting neural crest-derived structures (teeth and face) and mesodermally derived structures (posterior cranium), using datasets comprising 985 crania and 632 dentitions. Two facial, two posterior cranial, and molar mesiodistal and buccolingual dimensions were included. Pairwise analyses were based on an average of ~500 individuals for whom we have both cranial and dental data. Phenotypic, genetic, and environmental correlations were estimated

using a maximum likelihood approach implemented in SOLAR-Eclipse.

Our results reveal significant genetic correlations between molar length and both anterior and posterior cranial dimensions, including the sagittal diameter of the foramen magnum ($RhoG = 0.35$) and the prosthion-nasion distance ($RhoG = 0.32$; both $p < 0.0001$), suggesting incomplete pleiotropy. We found no evidence of environmental correlations among traits, indicating that shared environmental influences do not explain the observed phenotypic associations. Additionally, there is no clear pattern suggesting that adult phenotypic correlations align with differences in embryonic tissue origin. These findings provide preliminary evidence of genetic integration between dental and cranial traits and offer new insights into the co-evolution of the primate skull and dentition.



Heterochronic shift in gene expression contributed to the evolution of vertebrate sensory system

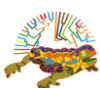
Sato S¹

¹Jichi Medical University

POSTER SESSION 3 - **P03.102**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Several different sensory organs and sensory neurons have arisen during vertebrate evolution. In the trunk region, cyclostomes have simple intramedullary mechanosensory neurons called Rohon-Beard (RB) neurons while amniotes possess complex extramedullary neurons in the dorsal root ganglia (DRG). Intriguingly, in the fish and amphibians, the sensory system of the trunk switches from RB neurons to DRG neurons during development. However, except the fact that RB and DRG neurons originate from neural crest or progenitor cells, the mechanisms underlying the phylogenetic transition and the ontogenic switching remain unknown. In *Xenopus*, overexpression of the SIX1 homeodomain protein promoted early apoptosis of RB neurons and the emergence of extramedullary sensory neurons, whereas knockdown of *six1* delayed the reduction of RB neurons. A sensory neuron-specific *Six1* enhancer identified in the mouse activated

transcription in *Xenopus* RB neurons earlier than endogenous *six1*. To confirm SIX1 function in intramedullary and extramedullary neurons was essentially unchanged, we analyzed mice deficient for *Six1* and paralogous *Six4*. The *Six1/4*-deficient embryos have intramedullary sensory neuron-like cells of neural crest origin and immature DRG neurons. The forced expression of SIX1 in the neural crest-lineage of *Six1/4*-deficient embryos partially rescued the phenotype. These results established the conserved role of SIX1 and suggested that the onset of SIX1 expression during sensory development in the amniote lineage shifted earlier after the divergence from amphibians. We suggest that the heterochronic shift in *Six1* expression contributed to the transition of trunk sensory differentiation and architecture, and hence to the evolution of trunk sensory system in vertebrates.



Parallel adaptation of tooth classes to dietary niches in Chiropterans

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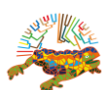
POSTER SESSION 3 - P03.136, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Mammalian tooth classes are considered a key innovation that has allowed mammals to colonize new dietary niches and diversify. From the conical shape of the earliest homodont teeth, mammals have evolved four distinct tooth classes (incisors, canines, premolars, and molars), each having a specialized morphology adapted to a specific function during food processing. While tooth classes are widely used in paleontology and for species identification, our understanding of the morphological signals that characterize the identity of each tooth class remains limited. To address this gap, we use the evolutionary radiation of Noctilionoid bats including Phyllostomids, as a model system. This superfamily is ideal to study these questions as these bats possess a complete mammalian dentition with four tooth classes and have radiated into nearly every mammalian ecological dietary niche (carnivore, frugivore, insectivore, nectarivore, omnivore, and sanguinivore) resulting in a huge variation in tooth number and shape.

We first characterized the precise morphological signature of two tooth classes, premolars and molars. To do so, we established a precise morphospace for each lower post-canine tooth across 151 species of Noctilionoid bats. We used a geometric morphometric approach incorporating two distinct sets of landmarks, 3 curves of semi-landmarks and 11 single-point landmarks,

for premolars and molars respectively. For both tooth classes and all teeth, we found that the species generally clustered by dietary niches, and separated along two main PCs that correspond to the height of the cusps and the width of the tooth. Interestingly, while some convergent patterns are observed for nectarivores and faunivores, stenodermatinae cluster in the same area of the morphospace indicating that their morphology retains a strong phylogenetic signal. When calculating disparity for each tooth, we found that premolars tend to have a significantly higher disparity than molars. Within tooth classes, we found an increase in disparity from the first molar m1 to the last molar m3 and that the middle premolar p3, when present, is more variable than p2 and p4, which is reminiscent of their timing of induction during development.

Together, these results reveal that premolars and molars evolved in tandem, exhibiting distinct but also overlapping patterns during their diversification. While they follow similar evolutionary trajectories in response to dietary adaptations, they exhibit clear differences that constitute a precise morphological signature for each tooth class, representative of tooth class identity. More broadly, these results provide insight about the diversification of repeated structures within and between classes.



Influence of Morphological Integration and Modularity on Evolutionary Rates and Disparity of Bird Beaks

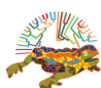
Ely R¹, Hipsley C¹, Sommer S¹, Nielsen R²

¹University Of Copenhagen, ²University of California

MEETING ROOM 118+119, August 21, 2025, 2:00 PM - 4:00 PM

The bird beak is a multifunctional innovation, having facilitated adaptations to a plethora of ecological niches, resulting in a grand evolutionary radiation currently represented by over 11,000 bird species. The sheer disparity of bird beak morphology implicates the role of evolutionary constraints stemming from magnitude (integration) and patterns (modularity) of trait covariance. We aim to evaluate potential associations between morphological integration and modularity on evolutionary rates and disparity at macroevolutionary scales in the avian bill. If integration and modularity act primarily to influence evolutionary constraints, the expectation is that rates and disparity should be negatively correlated with the degree of integration, and it is commonly posited that a positive association between modularity and rates and disparity exists. To explore these expectations, we leverage the vast taxonomic scale of the MarkMyBird dataset, represented by 3D semilandmarks along the culmen and tomia of 8748 avian species. All analyses were performed using phylogenetic comparative methods, employing a new avian supertree whose higher-level relationships are heavily weighed in favor of a relatively new and highly resolved phylogenomic avian tree. We performed analyses of morphological integration using the standardized effect size of relative eigenvalue variance and estimated evolutionary rates and disparity under Brownian Motion (BM) assumptions. Disparity was estimated using a special reinterpretation of the common BM evolutionary rate (σ^2), noting that standard functions to estimate σ^2 essentially yield estimates of phylogenetically transformed trait variance if the phylogeny is scaled to unit height a priori. Modularity was assessed by using the effect size of the covariance ratio, under the assumption that two modules drive the covariance structure of the bird beak consistent with numerous evo-devo studies. Modular analyses require a priori specification of

individual landmark assignments to hypothesized modules, as such we used Ward's linkage dendrogram based on landmark correlation matrices to detect most likely landmark assignments to modules. Evolutionary rates, disparity, integration, and modularity were analyzed per taxonomic order. Using phylogenetic generalized least squares, disparity and rates were positively and significantly correlated with the magnitude of morphological integration, such that higher rates and greater disparity are associated with greater trait integration (rates: $R^2=0.174$, $p=0.013$; disparity: $R^2=-0.392$, $p=1e-4$). The magnitude of modularity and rates and disparity were not significantly correlated (rates: $R^2=-0.038$, $p=0.82$; disparity: $R^2=-0.039$, $p=0.88$). The apparent positive association between rates, disparity, and integration is unexpected, counter to intuitive expectations that greater integration results in greater evolutionary constraints, thus resulting in limited morphological disparity and lower rates. Such relationships between integration and rates and disparity have been shown in other clades, falling under theoretical expectations of the 'facilitation hypothesis' and evolutionary lines of least resistance. Beak modularity does not appear to impact rates or disparity, indicating that either alternative modular hypotheses regarding the number of modules may need to be evaluated, or that the modular architecture and magnitude across avian orders remains invariant while the degree of overall integration is allowed to vary, consistent with expectations from mammalian modularity and integration studies. Indeed, across all avian orders the two-module landmark scheme estimated via Ward's linkage dendrogram consistently retrieves a ventral module including the beak tip, and a dorsal module composed largely of nasal traits. Such a pattern is qualitatively consistent with classic evo-devo studies of finch beaks, showcasing its potentially broadly conserved phylogenetic presence.



Neural crest biology shapes evolutionary dynamics of the lacertid skull across evolutionary scales

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MEETING ROOM 118+119, August 21, 2025, 2:00 PM - 4:00 PM

Background: The contrasting pleiotropic properties of the neural crest cells and the mesoderm, from which vertebrate skull bones are derived, likely modulate variation that is available to selection and thereby influence head shape evolution. Whether and how the influence of the dual developmental origin of the skull on evolution lasts beyond the population level and contributes to species diversification remains to be established.

Material and methods: We conducted 3D morphometric analyses with X-ray microtomography (CT) scans of lacertid skulls at two evolutionary scales. At the microevolutionary scale, we focused on the common wall lizard, *Podarcis muralis*, exhibiting a suite of exaggerated traits partially developing from neural crest cells, which has been termed the nigriventris syndrome. At the macroevolutionary scale, we compared 174 species of the lacertid family (44% of the described species).

Results: In the common wall lizards, we identified two modules of phenotypic covariance corresponding to the neural crest- and mesoderm-derived bones. The

neural crest module contributed more than the mesoderm to the differences in skull shape between the lizards exhibiting the nigriventris syndrome and the specimens with the ancestral phenotype. At the level of the lacertid family, we also observed modularity corresponding to neural crest- and mesoderm-derived bones. However, the neural crest derived module showed slower evolutionary rates and less disparity.

Conclusion: The primary importance of the neural crest for the evolution of the nigriventris syndrome at the population level contrasts with the macroevolutionary patterns in lacertids that suggests a more limiting role of the neural crest in diversification at the family level. We suggest that developmental properties of body parts, like those emerging from the neural crest-mesoderm duality generate stable covariance structures and influence evolvability across evolutionary timescales. However, the realized variation within this covariance structure may vary across levels, depending on external factors like correlational selection, which complicates views on the consistency between micro- and macroevolution.



Prenatal Hormones Shape Maternal–Fetal Morphological Integration in a Model of Cephalopelvic Covariation

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MEETING ROOM 118+119, August 21, 2025, 2:00 PM - 4:00 PM

The risk of mismatch between fetal head and maternal birth canal in humans is supposed to be greatly reduced by the presence of morphological co-variation between these traits. Neonates that inherit a large head size from their mother are less likely to obstruct labour provided that their mother also possesses a comparably wide birth canal. This high degree of morphological covariation in humans reflects an evolutionary compromise between the need for a large birth canal to accommodate large neonates and the need for a narrow canal to support pelvic floor muscles and enable efficient bipedal locomotion.

Recent studies have shown that this pattern of covariation between maternal pelvis and fetal head is also present in macaques, indicating that it is not uniquely human but may characterize other primates that birth large offspring.

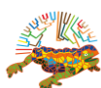
Here, we extend this line of inquiry to a non-primate, quadrupedal species that gives birth to relatively small neonates: the common house mouse (*Mus musculus*). Using geometric morphometrics, we examined within-individual covariation between skull and pelvic morphology across several wild-captured and laboratory mouse strains. Our results reveal significant covariation between neurocranial size and pelvic shape, with larger skulls associated with wider and rounder pelvic canals, and smaller skulls associated with narrower ones. Strikingly, the pattern of skull–pelvis covariation in mice mirrors that observed in humans, suggesting a conserved developmental mechanism across mammals, independent of neonatal size or locomotor mode.

To investigate potential developmental mediators of this integration, we tested whether prenatal sex steroids influence the

relationship between maternal pelvic shape and fetal head morphology. Pregnant C57BL/6 mice were treated with exogenous estrogen or testosterone between embryonic days E12.5 and E17.5, a critical window for skeletal development. We then measured covariation between maternal pelvic shape, fetal head shape, and fetal size across treatment groups.

Hormone exposure significantly alters patterns of skull–pelvis covariation. Both estrogen and testosterone treatments modified the degree and direction of integration between maternal and fetal morphology, indicating that prenatal hormonal environments can modulate the developmental architecture underlying cephalo–pelvic covariation.

Together, these results challenge the prevailing view that skull–pelvis covariation evolved solely in response to the obstetric constraints of large-brained primates. Instead, we show that this integration is present in a quadrupedal species with small neonates and may reflect a more general principle of coordinated morphological development. Moreover, our hormone manipulation experiments highlight a possible mechanistic link between endocrine signaling and the establishment of intergenerational morphological integration during pregnancy.



Reconciling stemness and specialization in neural crest cells drives avian beak diversification

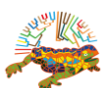
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MEETING ROOM 118+119, August 21, 2025, 2:00 PM - 4:00 PM

Multicellular development must balance organismal integration with tissue differentiation. Migratory stem cells often fulfill these dual needs by orchestrating region-specific tissue differentiation, yet how they balance stemness with positional specificity is unclear. Using novel analytical approaches capable of tracing millions of cells throughout avian beak development, we discovered a transient phase of "local mimicry" in migratory neural crest cells in which they briefly match protein profiles of the overlying epithelium. This "local mimicry" phase temporarily extends region-specific epithelial signaling, creating distinct boundaries that trap later arriving neural crest cells and seed localized cell condensations. Accumulation and

mixing of cells in these condensations lead to their morphological and molecular homogenization, erasing initial regional differences impaired by epithelial protein matching, thus restoring their multipotency and priming site-specific differentiation. These cycles of transient specialization and uniformity – driven by universal processes of cell proliferation and migration – allow neural crest cells to interpret evolutionarily conserved mesenchyme-epithelial signaling in a region-specific manner without compromising their stemness. By reconciling global integration and local specialization and enabling modular recombination of regulatory circuits, this developmental organization facilitates the remarkable evolutionary diversification of avian beaks.





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S10 - Eco-evolutionary dynamics driven by mobile genetic elements



Disentangling the complex network of phage-host interactions at genome resolution in freshwater

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POSTER SESSION 1 - **P01.129**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Bacteriophages are the most abundant and genomically diverse biological entities on the planet.

Bacteriophages and their dependence on their bacterial host has defined the evolutionary trajectory of both parties for millennia. Additionally, bacteriophages are an important contributor to gene flow in the environment through horizontal gene transfer. Bacteriophage genomes are constantly reshaping due to mutations and through their role in horizontal gene transfer which is consequently affecting their host range and impact on bacterial community. Phages also exert top-down control on dominating bacteria, influencing the species equilibrium dynamics and nutrient cycling in the surrounding ecosystem. High-throughput metagenomic studies have elucidated the vast diversity of phages in aquatic ecosystems including both marine and freshwater environments. However, the depth of characterization is greater for marine phage communities currently. Freshwater ecosystems have complex seasonal and vertical profiles throughout the year, changing in temperature and nutrient composition and housing microbial communities that are distinct from marine ones. This spatial and temporal diversity of phages and their direct impact on bacterial community dynamic in freshwater ecosystems is not yet fully characterised.

Freshwater ecosystems host a diverse community of phages, however, we cannot confidently assign phage to their respective host based on indirect linkage from

metagenomic data alone. Current metagenomic methods fail to resolve the complex nature of phage-host interaction networks and dynamics, undermining our ability to disentangle ecological relevance and evolutionary impact of phages in freshwater environments. With a lack of cultured references, we need to couple multiple high-throughput techniques in order to resolve a confident phage-host interactions network at community level.

In this study, we are collecting samples with high temporal resolution (twice a week for six consecutive weeks) from freshwater Lake Erken in Sweden. Collected samples were concentrated in different size fractions of 0.1 µm for bacteria and 30kD for viruses using tangential flow filtration. The concentrated bacteria and virus fractions were then exposed to each other to induce phage-bacteria interactions. These incubations were then filtered, and the collected biomass was used for short read Illumina and long read Pacbio sequencing to reconstruct high quality phage and bacterial genomes. In addition to in-silico host designation methods, in this study we will apply high-throughput single cell genomics to the incubations to resolve phage-host interactions and generate a confident network of phage-bacteria interactions. This confident network of phage-host interactions will enable a detailed understanding of the impact of phages in the flow of genetic material among freshwater bacterial community.



Diverse patterns of transposable elements expressions in *Macaca mulatta* and the regulation of gene expression in adjacent genes by tissue-specific TEs

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POSTER SESSION 1 - **P01.118**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Transposable elements (TEs) consist of approximately half of the primate genomes and may affect gene regulation in various ways resulting in the evolution of phenotypic diversity. Recently, it has become obvious that TEs play an invaluable role in physiological and pathological processes. Since TEs have regulatory motifs, they can act as regulators of gene expression. However, their expression and regulation across tissues are not clear.

Here, we analyzed TEs expression in 13 different tissue types of the rhesus macaque (*Macaca mulatta*), the most widely used non-human primate model as a human replacement, of a public RNA-seq dataset to investigate their participation in gene regulation and difference in each tissue. We also analyzed Cap analysis gene expression (CAGE) datasets of 14 different tissue types to attain CAGE transition start sites (CTSS). We show that TEs are not randomly distributed in the rhesus macaque genome, but are enriched differently depending on their genomic location. Most TEs in intergenic regions are silenced, and highly

expressed TEs are remarkably enriched in the thymus. Not only are four clusters of TEs found in various tissue types, but enrichment patterns are followed by tissue types and age of TEs. Particularly, 365 and 269 short interspersed nuclear elements (SINEs) and long interspersed nuclear elements (LINEs) are highly enriched in transcription start sites (TSSs), respectively, which may be potential regulatory activities. Additionally, we found 37 and 1226 tissue-specific TEs that are highly expressed on coding DNA sequence (CDS) and upstream, respectively, which may drive the overexpression of adjacent tissue-specific genes.

In summary, we present diverse patterns of TEs in rhesus macaques across 13 different tissues. Based on the analysis of pairwise comparisons and association between TEs and genes, we identified tissue-specific TEs in CDS and upstream regions where their nearby genes were also highly expressed. Our study will improve the understanding of TEs as regulators of nearby gene expression in a tissue-specific manner in primates.



How TEs dynamics follow lifestyle transitions in gall wasps (Hymenoptera: Cynipoidea)

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POSTER SESSION 1 - **P01.121**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Transposable elements (TEs) are selfish genetic elements that can replicate themselves and cause mutations by inserting into different loci in the host genome. TE insertions may also lead to non-allelic homologous recombination (NAHR), creation of novel regulatory sequences, and epigenetic changes. TEs inserted into introns interfere with the normal splicing pattern of precursor mRNAs, triggering various forms of selective splicing. For instance, insertion of Tgmt-CACTA TE into intron 1 of the Flavonoid 3'-hydroxylase gene in soybean which produced the t* allele, the gene that determines gray trichome color. In the British peppered moths, a TE insertion in the intron of the cortex gene increases its expression, leading to an alteration in the moths' body color.

The ability to form galls is a convergent trait in insects that has evolved independently several times in different orders. Gall wasps (Hymenoptera: Cynipoidea) comprise many species inducing galls on several host plants and, in addition to true gall inducers, they also consist of phytophagous inquiline, which live inside the gall tissue induced by gall wasps or other insects and parasitoid species. Cynipoidea species show an important variation in genome size (from 150 Mbp to up to 2.5 Gbp) highly correlated with differences in TE content. Noticeably, we

found that genome size correlates better with lifestyle, namely gall inducer, inquiline and parasitoids, than with phylogeny. We therefore investigated how TE dynamics could have played a role in or be influenced by the transition toward different lifestyle in Cynipoidea.

Twenty-nine species were selected to represent the different lifestyles and main clades of Cynipoidea. De novo detection and annotation of TEs was performed using a custom pipeline.

A total of 56,237 consensus sequences for repeats were reconstructed and our data suggest that intron size in Cynipidae appears to correlate with the number of transposons, with Gall inducers having a larger average intron size compared to Inquiline. Notably, Tc/Mariner elements seem to have played a particular role in intron size change in the different life styles, the gall inducing species having the largest introns. We have yet to determine if these convergent changes are the result of independent invasion of different families or horizontal gene transfer of the same family. We will also analyze how the change in intron size could have affected the expression of particular genes involved in gall induction.

Key words: Transposable element, Cynipidae, life lifestyles, convergent invasions, Gall inducers, Inquiline.



Mechanisms of infection in freshwater bacteria–phage systems

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POSTER SESSION 1 - P01.126, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Viruses are typically considered to outnumber their microbial hosts, often by an order of magnitude, particularly in productive freshwater lakes. Through their top-down control on bacterial populations, phages influence not only microbial dynamics and evolution but also biogeochemical cycles. While high throughput metagenomic approaches have revealed a vast diversity of phages specifically in aquatic ecosystems, they are inefficient in connecting these phages to their respective host and revealing their infection strategies. Cultured phage-host systems bypass this limitation, but they remain biased by the great plate count anomaly themselves. Majority of ecologically relevant freshwater bacteria are still very cumbersome to culture and their interaction with their hosts remain enigmatic.

In this study, we developed a culture collection from the eutrophic Lake Ekoln in Sweden. Samples were collected monthly from August 2024 to February 2025 (five time points in total), and dilution-to-extinction cultures were established using modified NSY medium. For each sampling point, six 12-well plates (72 wells total) were inoculated with ~1 cell. Success rates varied by season, ranging from 2.7% to 38%, and overall, approximately 40% of wells were successfully cultivated, yielding 113 positive cultures.

Each isolate is undergoing three rounds of dilution-to-extinction to ensure axenic and clonal cultures. When cultures are purified and stable, their taxonomy is assigned using Sanger sequencing and universal primers 27F and 1492R, targeting 16s rRNA gene. We have currently characterized isolates from August, September, and October. Cultures from August and September were primarily affiliated with the genera *Limnohabitans*, *Flavobacterium*, and *Polynucleobacter*, while those from October were dominated by members of the genus *Verrucomicrobium*.

Two of the isolated bacteria, affiliated with the ubiquitous genus *Polynucleobacter*, were selected for phage isolation. For phage enrichment and isolation water collected from the lake was pre-filtered through a 42 µm mesh on-site to remove larger plankton, then further filtered through a 0.1 µm filter in the lab to obtain a phage-containing flowthrough. This flowthrough was added to mid-exponential phase *Polynucleobacter* cultures to introduce phages. Phage infection was screened using flow cytometry comparing infected and control treatments. We use this phage-host system to perform gene resolved differential transcription analyses along the phage infection cycle to resolve the infection strategies and molecular mechanisms of freshwater phage–host interactions



Plasmid recombination: a key strategy for understanding the dynamics of plasmids and antibiotic resistance in bacteria

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POSTER SESSION 1 - **P01.131**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Antibiotic resistance (AR) is one of the greatest threats for public health. AR genes widely circulate through genetic exchange between bacterial individuals, mainly by being carried by mobile genetic elements such as plasmids. Because of their mobility between bacterial cells, plasmids can be seen as genetic entities with their own evolutionary trajectory, partly independent of that of their host. To understand AR gene dynamics, it is therefore essential to study the eco-evolutionary dynamics of plasmids. The two plasmid strategies mainly studied are their capacity for transmission either horizontal (from one cell to another, independently of their replication) or vertical, (from replication, and facilitated by the carriage of accessory genes (e.g. the AR gene) that may be beneficial to their host). However, these approaches consider plasmids as stable genetic entities, whereas there is growing evidence that they can shuffle genes when they coinfect the same bacterial cell. In our study, we will therefore consider the evolution of a new plasmid trait, that of their recombination rate. Based on a theoretical modelling approach, we propose to test the hypothesis that genetic shuffling may increase plasmid flexibility and enable them to cope with environmental fluctuations.

By transposing host-pathogen models from epidemiology, we developed a dynamical model of plasmids transferring horizontally and vertically within a bacterial population. The model is multiscale, with (i) AR genes that are carried by plasmid (ii) plasmids that belong to a given recombining family (iii) and bacteria competing for a limited resource in a population exposed to a fluctuating environment (antibiotic stresses). When two related plasmids (i.e. belonging to the same

family) coinfect the same bacterial cell, they would exchange genes with a given recombination rate, which represents the family strategy.

Our results show that over a very wide range of conditions tested, an intermediate recombination rate optimally buffers environmental fluctuations, enabling the genotype adapted to the current condition to be generated rapidly. There is a balance between getting rid of AR genes when there is no stress, but not too fast so that they can be remobilized rapidly when needed under antibiotic stress. An intermediate recombination rate confers a selective advantage by maintaining a diversity of plasmid genotypes, including those that are not adapted to current conditions, and are maintained at low densities but are likely to re-emerge when they become the most advantageous again (for example, during a period of stress). In this sense, our work shows that the spread of AR genes is highly dependent on plasmid recombination rate strategies.

The predictions of our models echo the growing body of work showing that the plasmids that pose a public health problem are extremely flexible and can mix genes at a not negligible rate when they coinfect the same bacterial cell. Our result suggest that the spread of AR genes is a matter of interaction between plasmids (or more largely mobile genetic elements), themselves and between plasmids and their bacterial host, alternating with conflict and cooperation. Mathematical modeling of plasmid evolutionary strategies combined with genomic analysis may constitute a powerful tool to disentangle these complex evolutionary interactions.



Purifying Selection Shapes the Dynamics of P-element Invasion in *Drosophila simulans* Populations

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POSTER SESSION 1 - P01.123, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Transposable elements (TEs) are DNA sequences that can move within a host genome. Many new TE insertions have deleterious effects on their host and are therefore removed by purifying selection. The genomic distribution of TEs thus reflects a balance between new insertions and purifying selection. However, the inference of purifying selection against deleterious TE insertions from the patterns observed in natural populations is challenged by the confounding effects of demographic events, such as population bottlenecks and migration.

We used Experimental Evolution to study the role of purifying selection during the invasion of the P-element, a highly invasive TE, in replicated *Drosophila simulans* populations under controlled laboratory conditions. Because the change in P-element copy number over time provides information about the transposition rate and the effect of purifying selection, we repeatedly

sequenced the experimental populations to study the P-element invasion dynamics. Based on the empirical data, we used Gaussian Process surrogate models to efficiently explore the parameter space and identify parameter combinations that best reproduce the experimental P-element invasion trajectories. Assuming that beneficial P-element insertions are negligible, we found that, in our experimental populations, 73% of new P-element insertions are under purifying selection with a mean selection coefficient of -0.056, highlighting the central role of selection in shaping P-element invasion dynamics.

This study underscores the power of Experimental Evolution as a tool for studying transposable element invasions, and highlights the pivotal role of purifying selection in regulating P-element dynamics. Furthermore, it also illustrates how computational modeling can complement experimental work to study TE invasions.



Repeatome analysis in an Amazonian riparian forest ground-herb reveal potential role of LTR-Retrotransposons on waterlogging adaptation mechanisms

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POSTER SESSION 1 - **P01.134**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The highly dynamic nature of riparian forests has led to a remarkable adaptability of the ground herb *Ischnosiphon puberulus* to a flooding gradient. Through an integrated genomic and transcriptomic approach, we uncovered the molecular basis of stress response of *Ischnosiphon puberulus* to thrive extreme flooding variability (from Igapo to Terra firme). This scenario of contrasting environmental conditions makes *I. puberulus* an ideal model for investigating the genomic bases of adaptive mechanisms in response to climate change. Long Terminal Repeat Retrotransposons (LTR-RTs) usually represent the most abundant fraction of plant genomes, mostly due to their replicative process, in which copies of active elements are constantly inserted into new genomic locations. The activity of LTR-RTs has the potential to generate genomic diversity by causing structural variation and/or impacting gene regulation and their continuing study has provided numerous insights into their role in shaping mechanisms of stress-related adaptation. Here, we aim to investigate the impact of LTR-RTs on the mechanisms of local adaptation of *Ischnosiphon puberulus* populations in a flooding gradient. To this end, we first used the reference *I. puberulus* genome, previously published by our research group, to annotate the LTR-RT elements at the lineage level using the DANTE_LTR pipeline. This analysis revealed that 29% of the *I. puberulus* genome is composed of LTR-RTs, with 4.4% belonging to the Ty1/copia superfamily and 23.8% to the Ty3/gypsy superfamily. The chromovirus Tekay (Ty3/gypsy) was by far the most abundant LTR-RT, accounting for 20.6% of the genome. We then used the TE-Density pipeline to estimate the average density of five major lineages of LTR-RTs (Ty1/copia-lvana, Ty1/copia-lkeros, Ty1/copia-Tork, Ty3/gypsy/chromovirus-CRM, and the

previously mentioned Ty3/gypsy/chromovirus-Tekay) that were located within and 10 kb (500 bp windows) upstream and downstream of *I. puberulus* annotated genes. The results showed an overall low average density of LTR-RTs within and in close proximity to genes, with increasing densities toward the 10 kb upstream and downstream regions. We also investigated LTR-RT density using the same parameters in a subset of stress-related genes. Previous work from our group identified expanded cytochrome P450 (CYP) transcripts with distinct expression profiles in populations under different flooding conditions. These enzymes are crucial for processes such as secondary metabolite biosynthesis, hormone metabolism, and stress resistance. The average LTR-RT density upstream of this subset of genes was up to five-fold higher than the average for the complete gene set, with no clear pattern throughout the 10 kb visualized region. The significantly different pattern of LTR-RT density in these important genes suggests that LTR-RTs may be involved in the expansion and/or regulation of stress-related genes, either by transposing additional copies or by affecting promoter/enhancer activity. Preliminary results showed that 20.5% of the identified LTR-RTs possessed intact target site duplications and primer binding sites, which are features indicating potentially active elements. As LTR-RT activity may still be shaping adaptive processes in *I. puberulus*, our next step is to use our available RNA-Seq data to evaluate which of these LTR-RTs are transcriptionally active and whether their expression differs among a sampling of 41 individuals across a flooding gradient. This expression data can then be integrated with our LTR-RT density results to assess whether LTR-RT activity is occurring near genes of interest and potentially contributing to adaptive responses on a genomic level.



Role of AluYRa1 insertion in Generating a New Isoform of the BHMT Gene in Old World Monkeys

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POSTER SESSION 1 - **P01.119**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background Transposable elements (TEs) make up approximately 55% of the human genome and are divided into retrotransposons and DNA transposons based on their mechanism of mobilization—copy-and-paste and cut-and-paste, respectively. These elements contribute to genome evolution and plasticity, often influencing gene regulation and alternative splicing (AS) by inserting novel splice sites. In our previous study, we identified a novel isoform of the BHMT gene in cynomolgus macaques containing an exon derived from AluYRa1 and Tigger 4 elements. This study explores the origin, insertion mechanisms, and evolutionary relevance of this isoform in primates.

Materials and Methods We examined BHMT gene structures in 18 primate species using NCBI and UCSC genome databases. Genomic PCR was performed on DNA samples from seven species to detect TE insertions, while in-silico PCR was applied to 11 additional species to trace evolutionary insertion events. To confirm the expression of the novel isoform, RT-PCR was conducted using total RNA from 10 tissues of cynomolgus macaques. Splice site prediction and sequence alignment were used to identify exonization patterns and truncations.

Results The canonical BHMT gene comprises 8 exons and 7 introns in most primates, except in gorillas, where a ninth exon is observed. In Old World Monkeys (OWMs), we identified that AluYRa1 retrotransposed into

a pre-existing Tigger 4 element between exons 7 and 8 via a target-primed reverse transcription (TPRT) mechanism. AluYRa1 provides a novel 3' splice site and Tigger 4 serves as a 5' splice site, enabling the formation of a new exon through exonization. RT-PCR analysis confirmed that this variant isoform is expressed in the cerebrum, heart, kidney, liver, and stomach. In-silico PCR revealed that a second AluYRa1 insertion, located upstream of the first, exists in four species. However, this second element underwent 3' truncation, likely due to incomplete TPRT. A short poly-T tail was observed following the target site duplication (TSD), indicating the presence of a poly-A tail in the RNA intermediate during Alu retrotransposition. Furthermore, four OWM species exhibit a ~40 bp deletion at the 3' end of AluYRa1, disrupting the splicing signal and preventing isoform formation.

Conclusion Our findings demonstrate that the combination of AluYRa1 and Tigger 4 elements can create novel splice sites that promote exonization and contribute to alternative splicing in a lineage-specific manner. These events are restricted to OWMs, as the AluYRa1 insertion is absent in great apes and New World monkeys. The observed truncation and deletion events further illustrate how subtle sequence changes in TEs can influence gene isoform evolution. This study underscores the significant role of TEs in generating transcriptomic diversity during primate evolution.



Sequence composition and conservation predict the phenotypic relevance of transposable elements

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POSTER SESSION 1 - P01.125, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Transposable elements (TEs) are powerful drivers of genome evolution, in part through their ability to rapidly rewire the host regulatory network by creating transcription factor binding sites that can potentially be turned to the host's advantage in a process called exaptation. However, the effects on the host phenotype vary widely among different TEs, and are in general poorly understood. In this work, we have classified human-mapping TEs based on their contribution to the human host phenotypes at both molecular and macroscopic scales. TE contributions to chromatin accessibility, gene expression, and the heritability of complex traits are strongly correlated to each other, confirming that the main mechanism through which TEs affect the host phenotype is through the rewiring of the regulatory network. TE sequence characteristics and evolutionary features are able to explain a large fraction of the variance in phenotypic relevance, and in particular

more than 50% of the variance of their contribution to the heritability of complex traits. A conspicuous exception to this pattern is represented by TEs of the ERV1 family, whose phenotypic impact cannot be explained by our models: In particular, this family includes a set of relatively young TEs whose phenotypic relevance is much larger than would be expected based on their sequence and evolutionary parameters. These TEs are involved in fast-evolving biological processes related to the interaction of the organism with its environment, a role that could in part explain their deviation from our models. In conclusion, our results confirm quantitatively that TE insertions affect the host phenotype mostly through the rewiring of its regulatory network; identify a signature of phenotypic relevance based on sequence and conservation properties; and highlight several TEs as promising candidates for functional studies.



Stealth plasmids: rapid evolution of deleted plasmids can displace antibiotic resistance plasmids under selection for horizontal transmission

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*POSTER SESSION 1 - **P01.133**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM*

Conjugative plasmids carrying antimicrobial resistance (AMR) genes are critical for the spread of AMR, due to their ability to transmit horizontally between bacterial hosts. We previously observed that during experimental evolution in the presence of abundant susceptible hosts, the AMR plasmid R1 rapidly evolves variants with increased horizontal transmission due to mutations causing increased plasmid copy number. Yet AMR was progressively lost from the evolving populations. Here, we show that AMR loss was associated with evolution of 'stealth' plasmids in which the AMR region is

spontaneously deleted, making plasmid carriage undetectable by plating on selective antibiotic-containing media. These plasmids transmit both vertically and horizontally more efficiently than the ancestral AMR plasmid, driving AMR extinction in bacterial populations and effectively acting as an intrinsic defence against AMR plasmids. Our results suggest that within-host plasmid evolution could be exploited to limit the spread of AMR in natural populations of bacteria.



The co-evolutionary dynamics of CRISPR-Cas and plasmids

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POSTER SESSION 1 - P01.136, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

CRISPR-Cas is an adaptive immune system that protects bacteria from virulent phages. Upon infection, CRISPR-Cas acquires memory against foreign invaders by storing spacers, which are sequences derived from invaders and are later used to recognize and degrade them. On a molecular level, CRISPR-Cas is also active against plasmids, important drivers of bacterial evolution. However, the impact of CRISPR-Cas on the ecology and evolution of plasmids is less understood compared to the well-studied interaction between CRISPR-Cas and phages.

Unlike phages, plasmids can be either beneficial or costly for their bacterial host. While some evidence suggests that CRISPR-Cas might allow the spread of beneficial plasmids, the interaction of costly plasmids and CRISPR-Cas is more complex. A simple mathematical model predicts that CRISPR-Cas can block the spread of costly plasmids. However, several escape mechanisms, such as anti-defense systems, enable costly plasmids to persist in bacterial populations despite CRISPR-Cas immunity.

For phages, it is well established that not only anti-defense systems but also escape mutations in immune targets allow CRISPR-Cas evasion. The role of escape mutations in maintaining costly plasmids under CRISPR-

Cas pressure is unclear. To investigate whether plasmid mutations promote persistence of costly plasmids in the presence of CRISPR-Cas we use mathematical modelling. Our stochastic model focuses on the early phase of plasmid and CRISPR-Cas co-evolution, where each CRISPR-Cas system carries at most one spacer. In the case of phages, this initial phase of co-evolution is known to often drive phages to extinction through population-wider spacer diversity.

While our preliminary results suggest that escape mutations might allow costly plasmids to persist through the initial co-evolutionary arms race between CRISPR-Cas and plasmids, this outcome may depend strongly on the effect the escape mutations have on plasmid fitness. CRISPR-Cas spacers often target plasmid backbone genes, which are likely harder to escape without affecting plasmid fitness. So far, we assumed in our simulations that escape mutations increase plasmid cost. By incorporating varying fitness effects of escape mutations including their impact on other plasmid parameters such as plasmid conjugation, our mathematical model will improve the understanding of the co-evolution between CRISPR-Cas and plasmids and the impact CRISPR-Cas has on plasmid spread.



The latent cis-regulatory potential of mobile DNA in Escherichia coli

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POSTER SESSION 1 - P01.124, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Transposable elements can alter gene regulation in their host genome, either when they integrate into a genome, or when they accrue mutations after integration. However, the extent to which transposons can alter gene expression, as well as the necessary mutational steps, are not well characterized. Here we study the gene regulatory potential of the prominent IS3 family of transposable elements in E.coli. We started with 10 sequences from the ends of 5 IS3 sequences, created 18,537 random mutations in them, and measured their promoter activity using a massively parallel reporter assay. All 10 sequences could evolve de-novo promoter activity from single

point mutations. De-novo promoters mostly emerge from existing proto-promoter sequences when mutations create new -10 boxes downstream of preexisting -35 boxes. The ends of IS3s harbor ~1.5 times as many such proto-promoter sequences than the E. coli genome. We also estimate that at least 26% of the 706 characterized IS3s already encode promoters. Our study shows that transposable elements can have a high latent cis-regulatory potential. Our observations can help to explain why mobile DNA may persist in prokaryotic genomes. They also underline the potential use of transposable elements as a substrate for evolving new gene expression.



Towards building a global atlas of microbial Mobile Genetic Elements

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POSTER SESSION 1 - P01.130, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The metagenomics revolution revealed the ubiquity of microbes across all known ecosystems on earth. It provided the methods to study taxonomic and functional microbial diversity. However, we still lack understanding about how microbes adapt and survive across the diverse habitats of our planet. As methods to study microbial adaptability fueled by horizontal gene transfer of molecular functions are limited. To address this, we focused on the identification of the drivers of horizontal gene transfer in microbes and microbial communities, the Mobile Genetic Elements (MGE) namely IS elements, transposons, phages, conjugative elements. By addressing a methodological gap in the field for simultaneous identification of different MGE types and their interactions at a go, we made complete MGE profiles of environmental microbial (meta)genomes accessible. Our knowledge-based framework used combination of universal marker gene-based approach and

pangenome information to identify >23 million MGEs across ~800k complete genomes and ~200k Metagenome Assembled Genomes from diverse host-associated and environmental habitats represented in public datasets. We identified MGE interactions represented by >4 million nested elements, and observed recent horizontal spread of full length MGEs transcending the prokaryotic tree of life. Despite the presence of strong MGE type specific genomic signatures, we found certain molecular functions promiscuously carried as cargo by different types of MGEs. Thus, we present a modular, scalable and adaptable computational framework that enables high resolution comparative investigations of MGEs, their interactions, cargo functions and horizontal spread at planetary scale. Our framework builds the foundations for addressing the knowledge gap of the role of MGE mediated horizontal spread in shaping microbial communities of our planet.



Transposable element mobilization in the soma of hypomethylated *Arabidopsis thaliana*

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POSTER SESSION 1 - **P01.128**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Transposable elements (TEs) are potent drivers of genome evolution through their capacity to induce heritable genetic changes. In animals, early germline specification limits the opportunity for somatic TE mobilizations to contribute to heritable variation. In contrast, plants exhibit a more plastic and late specification of the germline, allowing somatic TE insertions, although rare, to potentially enter the germline and be inherited.

Prior studies in *Arabidopsis thaliana* have largely centered on TE mobilization in the germline of DNA hypomethylation mutants and somatic transpositions remain largely underexplored.

Here, we used high-accuracy PacBio HiFi long-read sequencing to investigate somatic TE activity in individual plants of a non-

reference *A. thaliana* strain lacking METHYLTRANSFERASE1 (MET1), a key enzyme in DNA methylation maintenance. We found that most mobile TE families overlapped with those previously identified in germline-focused studies, yet mobilization often involved distinct element copies. Our approach also uncovered active TEs absent from standard annotations. Somatic TE activity varied between individuals and across TE families. Notably, we identified signatures of alternative transposition involving two closely spaced Mutator-like elements, suggesting a potential mechanism underlying localized genomic instability.

These findings highlight somatic TE mobility as a powerful, rapid readout for studying TE behavior at single-organism resolution and offer new insights into the genomic consequences of epigenetic deregulation.



Unveiling Hidden Phage Diversity: Addressing Assembly and Identification Biases in Metagenomic Analyses of Mutation Hotspots

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POSTER SESSION 1 - **P01.127**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Standard metagenome assembly pipelines often struggle to recover repetitive, small, highly diverse, or rapidly evolving sequences. This leads to fragmentation of the assembly and can lead to underrepresentation of genomic regions important for phage evolution and host interaction. In this study, we explore how tracing identified viral contigs in assembly graphs and identifying assembly bubbles can help uncover hidden viral diversity typically missed in metagenomic studies of virus diversity.

In this study, we analyzed eleven publicly available metagenomic datasets from Lake Erken, Sweden, to evaluate the extent of viral sequence recovery across successive rounds of assembly. Each dataset was assembled using MEGAHIT. Metagenomic reads were mapped against assembled contigs larger than 2 kb. The unmapped metagenomic reads were extracted, normalized with bbnorm, and reassembled. This process was repeated 2 times. The first round of assembly produced over 446,000 contigs (2–329 kb size range), and an additional 17,900 (2–8 kb size range) and 378 (2–3.4 kb size range) contigs were recovered in the second and third assembly rounds, respectively.

Viral contigs were identified using a combination of geNomad, VIBRANT, and PPR-meta. Within all assembled contigs, 65,286 contigs with an overall size of 285 mb were identified as viral. These viral candidates were then traced to their corresponding positions in assembly graphs to detect structural patterns such as bubbles and super bubbles. We recovered 13,562 graphs containing at least two viral nodes. These virus-affiliated assembly networks had a total size of 855.19 mb, from which only ~20% (175.75 mb) was identified as viral sequences using state-of-the-art virus identification tools. Observed graph structures suggest the presence of complex sequence variation, which is relevant to population diversity, pangenome-level organization, and host interaction.

We aim to further investigate how such graph features relate to mutation hotspots and strain variation, particularly in genomic regions often fragmented or excluded in metagenome assemblies. If successful, this approach could offer a scalable workflow to enhance viral genome recovery and improve identification strategies in complex microbial communities



A phage protein evolved to limit satellite phage induction and mobilization

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MEETING ROOM 122+123, August 18, 2025, 2:00 PM - 5:00 PM

Streptococcus thermophilus is one of the most widely used lactic acid bacteria in the production of various cheeses and yogurts. However, even if carefully selected, industrial strains can be infected by virulent phages naturally present in milk, leading to lower-quality fermented products. Developing tools that harness different phage resistance mechanisms could help reduce production losses and associated costs. *S. thermophilus* relies heavily on the CRISPR-Cas system to defend itself against phage infections. Nevertheless, some phages have evolved strategies to bypass this defense, such as producing anti-CRISPR (ACR) proteins that bind directly to Cas9 and deactivate the system. The overall aim of this study is to identify alternative natural methods to enhance phage resistance in *S. thermophilus*, since the use of genetically engineered organisms is generally viewed negatively by consumers.

Mobile genetic elements (MGEs) play a crucial role in bacterial evolution by expanding the gene pool within bacterial populations. Satellite phages are a distinct subset of MGEs, found integrated into the genomes of various bacterial species, including *Staphylococcus aureus*, *Escherichia coli*, and *Vibrio cholerae*. Previous studies have shown that specific satellite phages in *S. aureus*, known as SaPIs, can interfere with the replication of certain temperate phages. In our analyses, we observed structurally similar satellite phages in the genome of 69% *S. thermophilus* strains. This high prevalence suggests that satellite phages may provide a selective advantage to *S. thermophilus*, a species known to harbor few or no prophage.

We recently showed that some *S. thermophilus* satellite phages (StCIs) can spontaneously excised and circularized from the bacterial chromosome as detected in overnight culture using PCR with primers targeting both ends of the StCI. Through CRISPR-Cas9 interference, we were also able to cure some *S. thermophilus* cells of StCI. Using these StCI-free strains, we successfully mobilized StCIs from one *S. thermophilus* strain to another via natural competence (ability to take up DNA from its environment). Of note, we found that exchanging specific StCIs enhance the overall phage resistance of the recipient strain. Here, we further investigated the phage resistance phenotype conferred by acquiring a new StCI.

We first isolated virulent phage mutants capable of bypassing the resistance phenotype. Genome analysis identified a single nucleotide mutation that led to an amino acid substitution in a DNA-binding protein. We also found that the wild-type phage DNA-binding protein was able to trigger StCI induction, while the mutated version could not. Further analysis revealed that this mutation imposed a fitness cost on the virulent phage, as evidenced by smaller plaque sizes compared to the wild-type. Finally, we demonstrated that StCI induction by a virulent phage enhances its mobilization via natural competence. However, this mobilization was almost entirely abolished in the presence of the phage mutant. Taken together, our findings highlight the rapid evolutionary adaptation of virulent phages to the StCI resistance mechanism. While the mutated phage protein prevented StCI induction and reduced its mobilization through natural competence, it did so at the cost of phage fitness.



A selective bottleneck during host entry drives the evolution of new plant symbionts

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MEETING ROOM 122+123, August 18, 2025, 2:00 PM - 5:00 PM

The acquisition of mobile genetic elements (MGEs) can trigger radical shifts in microbial lifestyles, for example by converting a mutualist into a pathogen or vice versa. These transitions often involve changes in host range, infection strategies, or ecological niches, which typically require specific adaptations to the different stages of the new life cycle. While comparative genomic studies have documented several MGE-associated lifestyle transitions, a detailed understanding of the tempo and mode of adaptation to new symbiotic lifestyles is still lacking.

Here, we sought to evolve new nitrogen-fixing bacterial symbionts of the legume plant *Mimosa pudica* from an ancestral plant pathogenic bacterium, *Ralstonia solanacearum*, which was engineered with a plasmid carrying essential symbiosis genes. We experimentally evolved this strain for over 900 generations during serial passages through a simplified symbiotic life cycle (comprising rhizosphere inoculation, root entry, and within-plant proliferation) and

characterized the dynamics of molecular and phenotypic adaptation in the evolved populations.

Multiple independent lineages exhibited rapid and parallel fitness increase during their interaction with *M. pudica*. Whole-population sequencing revealed a continuous accumulation of mutations, facilitated by a plasmid-induced transient hypermutagenesis phase before host entry. We observed sequential sweeps of mutational cohorts, with multiple adaptive mutations co-occurring within these cohorts. Notably, all adaptive mutations enhanced host entry, while only a subset also improved within-host proliferation. Computational modelling suggests that this pattern is a consequence of the strong selective bottleneck occurring at host entry and immediately following the hypermutagenesis phase. Our findings highlight how selective bottlenecks shape adaptive trajectories of symbiotic bacteria experiencing complex infection processes.



Bacterial warfare plasmids are associated with virulence and antimicrobial resistance

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MEETING ROOM 122+123, August 18, 2025, 2:00 PM - 5:00 PM

Bacteria have evolved diverse weapons to kill or inhibit competitors. These weapons are often encoded on large mobile genetic elements that encode additional genes and functions. However, how these mobile genetic elements evolve and their impacts on the ecology and evolution of bacterial communities remains poorly understood. Here we explore this question using the bacteriocins of *E. coli*, which are both well studied and have large well-annotated genomic resources. While bacteriocins are found widely across *E. coli* strains, we find that carriage is particularly associated with pathogenic extra-intestinal (ExPEC) lineages, including many clinically-important pandemic strains. These pathogenic strains commonly carry large plasmids that encode the bacteriocins but also virulence factors, including siderophores, and antimicrobial resistance mechanisms. Notably, combinations of siderophores and

bacteriocins targeting siderophore receptors have evolved independently multiple times and are maintained in certain lineages, likely enhancing bacterial survival in complex environments like the inflamed human microbiome. Across all *E. coli* strains, we identify numerous orphan immunity proteins, suggesting that bacteriocin-mediated warfare plays a key role in shaping microbial communities. We also present evidence for frequent horizontal transfer of bacteriocin genes between strains via plasmids and between plasmids via transposons. Finally, several *E. coli* bacteriocins are shared with *Salmonella enterica*, reinforcing their link to virulence. Our findings highlight the role of *E. coli* bacteriocins in defining bacterial communities and as potent antibacterial weapons in antimicrobial-resistant pathogens.



Evolutionary dynamics of phage domestication across prokaryotes

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MEETING ROOM 122+123, August 18, 2025, 2:00 PM - 5:00 PM

Mobile genetic elements (MGEs) drive multiple functional innovations within microorganisms. Notably, it has been hypothesized that prophages were domesticated into contractile secretion systems driving inter-bacterial warfare, such as the Type VI secretion systems (T6SS). We setup to identify the complex processes of phage co-option and functional innovation leading to domesticated contractile systems across the available diversity of complete prokaryotic genomes.

To construct our dataset, we integrated different methods using sequence and structural similarity to search for distant homologs of experimentally characterized systems (tailocins, external contractile injection systems, T6SS). We detected more than 20,000 systems and related phages across bacteria and archaea, redefining their distribution. To reconstruct their most probable path of evolution, we build phylogenetic trees using a variety of

techniques with tree search based on sequences (maximum likelihood and Bayesian sampling) or structures. Our results show that contractile systems have been co-opted from phages numerous times, particularly in the case of tailocins. We refined the phylogenies of each systems clade and identified common aspects between independent paths of prophage domestication.

These results shed light on the long-term evolutionary impact of MGEs in prokaryotes. Of note, contractile injection systems enhancing host fitness by antagonising microbial competitors are sometimes encoded in MGEs and thus transferred across bacteria and archaea. Together, these results underline the importance of mobile genetic elements in microbial interactions. On one hand they promote functional innovations, on the other hand they spread these innovations across microbial lineages.



Genetic exchange networks bridge mobile DNA vehicles in the bacterial pathogen *Listeria monocytogenes*

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MEETING ROOM 122+123, August 18, 2025, 2:00 PM - 5:00 PM

Background: *Listeria monocytogenes* (Lm) is an opportunistic foodborne pathogen responsible for gastrointestinal illnesses and life-threatening invasive infections. Antimicrobial resistance, virulence, and the ability to rapidly adapt to stressful environments in Lm lie in part on its mobile genetic elements (MGE).

Materials and methods: Here, we aim to characterize the MGE pool of a clinical Lm population using 936 genomes sampled across New York State, USA. We built a network based on sequence homology among putative MGEs.

Results: Within the network are communities of densely interconnected MGEs indicating high genetic similarity in their DNA regions. Although most connections involve the same MGE type, subsets within the network link different MGE types (plasmid-transposon, phage-plasmid). Phages and transposons did not share any genetic connections, suggesting impermeable barriers of exchange between them. Genes involved in stress tolerance are overrepresented in plasmids and transposons, and are mobile between vehicles. Analysis of long-read sequences of a subset of our dataset (n=37)

and publicly available, globally distributed complete genomes (n=425) recapitulated the MGE connections we observed. Our findings reveal a structured but interconnected network of genetic exchanges between different mobile DNA vehicles.

Conclusions: The impact of genetic exchanges among MGEs is threefold: (i) large variation in genetic content among distinct co-circulating lineages; (ii) adaptive potential that can be rapidly disseminated at short evolutionary timescales; and (iii) genetic mosaicism of DNA vehicles that lead to the confluence of traits, especially those relevant to human health and disease. Altogether, our findings add an extra layer of complexity in the forces governing HGT in Lm that extend beyond the transfer between donor and recipient cells. More generally, mosaicism in MGE illustrates the interconnected evolutionary histories of a priori distinct entities. Our work provides a portrait of the standing diversity of MGEs in clinical Lm, which is useful in understanding the bacterial genetic and evolutionary features that make Lm a highly resilient and persistent pathogen in various environments.



Into the Evolutionary Arms Race: Role of Mobile Genetic Elements in Giant Viruses

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MEETING ROOM 122+123, August 18, 2025, 2:00 PM - 5:00 PM

Eukaryotic giant viruses are a highly diverse group of recently discovered viruses classified within the phylum Nucleocytoviricota. They exhibit remarkably large genome sizes, believed to result from gene duplication and horizontal gene transfer facilitated by genetic exchange among eukaryotic hosts, viruses, and bacterial endosymbionts. Giant viruses also harbor prokaryotic-like mobile genetic elements (MGEs), such as group I introns and inteins associated with homing endonuclease domains, which promote MGE mobility and genome evolution. Although MGEs have been identified in giant viruses, their specific roles remain unexplored. Here, we investigate whether MGEs confer fitness advantages to giant viruses during co-infections within amoeba host cells. Using homologous

recombination, successive knockout mutants of MGEs in *Acanthamoeba polyphaga* mimivirus (APMV) have been generated. The impact of these deletions on viral fitness and competitive ability was assessed by comparing the replication success of mutant and wild-type viruses, as well as by visualizing the cellular localization of competing viruses via fluorescence microscopy. Preliminary results show a significant decrease in the fitness of the knockout mutant in presence of the wild-type virus, suggesting that the MGE confers a competitive advantage. This research aims to elucidate the role of MGEs in the evolutionary arms race among giant viruses, other parasites, and their hosts, thereby advancing our understanding of ecological dynamics within host-associated microbial communities.



Rapid genetic differentiation across short geographic scales in firefly *Photinus pyralis*

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MEETING ROOM 122+123, August 18, 2025, 2:00 PM - 5:00 PM

North American fireflies have experienced recent shifts in selective pressures due to human encroachment, habitat destruction, pesticide use, and light pollution. The ability of organisms to adapt to these environmental shifts depends directly on the substrate of mutations available to serve as targets of selection. Different mechanisms of mutations, duplications, TE movement, rearrangements or SNPs, appear at different rate with differing impacts on selective coefficients. If evolution is limited by mutation, then targets of selection will be constrained to standing genetic variation. However, if mutations appear rapidly, then organisms can adapt more freely with adaptive solutions from new mutation. We have identified two populations of fireflies *Photinus pyralis* in close proximity less than 11km apart with phenotypic differences in light signal color. We use a whole genome population genetic approach to identify targets of selection that also show strong differentiation across populations beyond

neutral expectations. Genes at signatures of adaptive differentiation include eye development genes, pigment processing pathways, and pesticide resistance loci. Both SNPs and tandem duplications appear to be mutation limited, with over 95% of targets of selection coming from standing genetic variation shared across populations. However, 35-40% of adaptive TE movement and rearrangements are derived from new mutations. These results indicate that transposable elements and rearrangements rapidly remodel genomes and fortuitously contribute to adaptive changes on short timescales. Our results suggest that TEs offer one mechanism to circumvent mutational constraints during adaptive walks resulting in rapid evolutionary change. These mutational mechanisms appear to be an important component of adaptation in fireflies deserving greater attention in the emerging field of non-model population genomics.



Too Hot to Keep, Too Cool to Skip: The influence of thermal environment on the diversity of transposable elements in Polychaets

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MEETING ROOM 122+123, August 18, 2025, 2:00 PM - 5:00 PM

Genome evolution plays a central role in enabling organisms to adapt to ever-changing environments, especially in the context of global environmental change. Among the primary drivers of genetic plasticity are transposable elements (TEs), which can induce various mutations as repeated and mobile sequences. To persist in genomes, TEs have evolved diverse strategies to evade host repression. One well-described mechanism is stress-induced transposition, whereby biotic or abiotic stresses trigger TE activation, leading to new insertion events. This makes TEs essential players in the adaptive response. Temperature, as an abiotic factor, is known to influence TE activity. In this study, we examine the role of thermal environments in shaping TE diversity at a macroevolutionary scale, using marine polychaete annelids as a model system. These mainly benthic organisms occupy a wide range of thermal habitats, from polar waters to hydrothermal vents and temperate coasts. They provide a unique opportunity to investigate the effects of temperature on genome evolution through a comparative genetic analysis. TE diversity varied substantially among polychaete species, with significantly lower diversity in

species exposed to extreme and unstable temperatures, associated with hydrothermal vents. Indeed, the diversity of most TE types was significantly reduced in such environments, while it was higher in thermally stable habitats. Only the diversity of tyrosine recombinase (YR) retrotransposons appeared to be more influenced by phylogenetic lineage, showing lower diversity in Sedentaria, though also affected by the thermal environment. Finally, phylogenetically informed analyses confirmed that both lineage and thermal environment shape overall TE diversity. Our results suggest a host response to the overall TE load—heavier in thermally unstable environments—rather than a TE-specific dynamic linked to temperature. In unstable environments, a high mutation rate induced by transposition may drive purging of TE diversity to maintain genome integrity. In contrast, species in stable environments retain diverse TE families, possibly preserving adaptive potential for future stress. These findings highlight long-term eco-evolutionary dynamics governing TE-host interactions and the role of the thermal environment in genome evolution.





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Do artificial intelligence tools help students with their evolutionary ecology homework? And are they willing to disclose it?

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POSTER SESSION 2 – P02.025, EXPO AREA, August 19, 2025, 5:00 PM – 7:00 PM

Background: Unanimous access to artificial intelligence (AI) tools is an opportunity for ease of work and creativity. Researchers and students use them. Are students using them effectively? Are they ready to disclose their use? So far, the first of those questions has not been addressed in the case of university students.

Materials and methods: Here, I used data from homework assignments in the Evolutionary Ecology course taught to MSc students over five years (2020-21 and 2023-2025), with the last three years characterized by the availability of AI tools. In 2025, an optional declaration of AI use was added to the assignments. In each year there were between 10 and 31 students, and here I have analysed over 250 of their assignments. In subsequent years, some homework assignments had the same content but different numerical values. In each case, the specific aspect of the homework can be rated as correct/incorrect. These are 1. estimating the population size required to detect inbreeding, 2. calculating the inclusive fitness of individuals helping their kin to reproduce, 3. determining the proportion of individuals at which the population could reach the mean maximum

fitness, 4. assessing the interaction term in a 2x2 factor simulation of the persistence of the metapopulation system.

Results: I found that the probability of correct answers was higher in two tasks and lower in two others before and after the introduction of AI tools. The reported use of AI tools varied widely across the four assignments, indicating that students do not use AI "by default" but rather when they feel the need. However, at the individual level, there was no relationship between correct answers and reported use of AI.

Conclusions: These results may indicate that students did not understand the nature of the questions or were unable to effectively prompt the AI for correct answers. It is less likely that the tasks were inherently resistant to AI assistance. Either way, these findings may be useful for other teachers of evolutionary ecology. Irrespective of the merits of the tasks, I was pleased to see that the majority of the students, when given the opportunity, filled in the questionnaire regardless of whether they reported using AI or not. This confirms previous findings that students are willing to use AI in an open and professional manner.



From predation to mutualism: authors' gender identities influence the focus of ecological research on species interactions

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POSTER SESSION 2 - P02.024, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The lack of inclusivity and diversity in evolutionary biology and ecology has been shown to affect the questions that are asked and the research that is published. Journals also play a critical role in either reinforcing or mitigating structural imbalances, and their editorial practices can strongly influence openness and representation across the field. A large proportion of studies in evolution and ecology focuses on species interactions, including competition, predation, and mutualisms, which play a central role in shaping natural systems and evolutionary dynamics. Despite the prevalence and importance of a wide variety of species interactions, recent reviews have revealed that ecology journals and textbooks are dominated by studies of negative interactions like competition and predation, with much less attention paid towards mutualisms.

Given that scientists' identities and backgrounds influence the scientific questions they pursue, we test the hypothesis that one mechanism for ecology's focus on negative interactions is the historical overrepresentation of men among published ecologists. We estimated

the gender of over 30,000 authors, based on their names, of approximately 9,000 papers published in 13 leading ecology journals over the past 25 years and found a strong association between author gender and the type of interaction studied with no influence of the author's geographical affiliation and the studied taxa. Women are significantly more likely to study positive interactions like mutualisms, whereas men are more likely to study competition and predation. This gender pattern holds across author positions but is even more pronounced among last authors. While women are not inherently more cooperative than men, they are often socialized to be, and socialized gender norms may influence scientific interests.

We further analysed how these patterns have shifted over time by extending our study to the past 50 years and found that as the number of female authors increases, so does the richness of interaction types. Our study provides original evidence that increasing diversity within scientific fields broadens our understanding of the natural world and contributes to a more inclusive and trustworthy evolutionary biology community.



Psychological insights for building accessible R Shiny apps in ecological and evolutionary modeling

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POSTER SESSION 2 - **P02.022**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Mathematical models are essential for managing environmental and health challenges, yet they can be notoriously inaccessible to students and researchers alike. A research gap exists where we know math is inaccessible, but we do not know exactly why. This project aims to enhance the accessibility to mathematical models in ecology and evolution by developing evidence-based strategies for designing and disseminating interactive web apps.

We are conducting a psychological study to identify app design features that best support bachelor student learning and motivation. Our hypothesis is that students who interact directly with an app will perform better at creating their own model than those watching a video demonstration of the same app. Moreover, we test whether a modular app design, which progressively reveals model parameters, will reduce cognitive load and enhance learning compared to a fully revealed version. We recruited bachelor students from universities with English-language instruction for a 20-minute online learning task and randomly assigned them to app treatments as well as one of three case studies in ecology, evolution, or infectious disease. Each case study uses mathematically similar models — dynamical systems with alternative stable states and bistability — enabling us to assess a

common set of learning outcomes; students will understand i) the consequences of multiple attractors for the final state of the system and how these are impacted by ii) different parameter combinations as well as iii) initial conditions. By comparing participants' responses to case study-specific scientific questions before and after the learning task, we identified interactive app features that can best support different students in becoming better mathematical modelers. Our findings will improve educational strategies and enhance communication of mathematical models among researchers.

Our long-term goal is to establish a public, community-sourced database of interactive apps in ecology and evolution, which showcases and promotes the evidence-based design principles identified by our study. This database will provide educators with a rich pool of open-source resources and improve accessibility to research findings, for both researchers and change-makers, by offering interactive tools for visualizing new models. Presenting our preliminary findings and future project plans at this venue will foster collaborations and trust within the community. We welcome feedback to ensure this database becomes a valuable, inclusive resource for all.



SPI-Birds: increasing open, reliable and transparent analyses based on avian long-term monitoring in the wild

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POSTER SESSION 2 - **P02.023**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Collecting long-term data on wild bird populations is historical in the field of ecology and widespread across the globe, providing valuable insights into evolutionary and ecological concepts. Yet, it seems there are as many ways to collect, enter and store data as there are existing datasets. Furthermore, for many datasets, metadata description is partial or lacking, drastically reducing the ability to reuse the collected data or to incorporate them in large-scale analyses. In addition, lack of metadata information on datasets from terminated projects may lead to data loss. To transition towards Findable, Accessible, Interoperable, Reusable (FAIR) data in avian evolutionary ecology, the Studies of individually-marked breeding birds (SPI-Birds) Network and Database (hereafter "SPI-Birds") was established in 2019 to offer a focused, centralized and well-defined grassroots community (Culina et al. 2021). SPI-Birds makes research groups and their datasets visible by enriching data with FAIR metadata, providing systematic data archiving, converting all datasets into a standard format and thus facilitating data sharing and comparative, cross-dataset analyses.

An important objective of the SPI-Bird project is to increase the transparency of the research lifecycle of the bird research community, including data processing and data analysis. SPI-Birds' core activities have expanded to encompass a work package dedicated to improving Open, Reliable and Transparent analyses related to SPI-Birds

data. The aim is to connect the SPI-Birds database to a library of data processing and analytical tools, and to implement a code peer-review process for which we provide guidelines. Codes qualified to be included in the SPI-Birds code library are fitted to data in SPI-Birds' standard format, ensuring the codes themselves are standardized to increase functionality across different datasets (i.e. populations) standardized to the SPI-Birds standard format. As part of the project, we will invite users of SPI-Birds data to share their analyses codes (large-scaled quantitative genetics, selection analyses, etc.) in our code library. We will provide guidelines and a code review process to enhance openness, reliability and transparency of the shared codes.

This talk will be an illustration of the efforts developed by the SPI-Birds network to provide practical tools aimed at improving the application of FAIR principles to ecological data along with open, reliable and transparent analyses. Besides presenting some instances of large-scale analyses that used the SPI-Bird standard format, I will also present the different tools we are implementing to reach our aims of open, reliable and transparent analyses.

Currently, SPI-Birds hosts 165 studies covering 329 populations on 85 different species of birds. The ESEB conference is also a perfect opportunity to strengthen SPI-Birds network and increase the range of studies included.



Career breaks and care-giving: challenges and opportunities for flexible working in evolutionary ecology

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MEETING ROOM 122+123, August 19, 2025, 2:00 PM - 4:00 PM

In biology, as in many other fields of research and society, we still face problems of gender equity (in progression and salary), and of women leaving the workforce. Some of this can be down to institutional barriers to progression, poor support during and after career breaks (e.g. maternity leave), and issues with culture. Here, we discuss our experiences of support before, during and after maternity leave and the impact this had

on our careers. We talk about flexible working patterns as a solution to support the return to work while balancing caring responsibilities, and our positive experiences of working part-time as academics. We recently set up a working group to discuss the challenges of being part-time and how institutions and individuals can better accommodate and support part-time workers.



Lessons learned from the COVID-19 pandemic on Open, reliable, and transparent practices

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MEETING ROOM 122+123, August 19, 2025, 2:00 PM - 4:00 PM

With the rapid emergence of variants with different transmission and antigenic properties, concepts and methods from evolutionary biology quickly became critical to studying the origin and spread of SARS-CoV-2, the virus causing COVID-19. The pandemic highlighted the need for open, reliable and transparent (ORT) practices for tracking SARS-CoV-2's evolution, adapting vaccines to these changes, and informing policy decisions. In this presentation, I propose to take a retrospective look at the challenges encountered, how they were

overcome, and whether lessons were learned – and remembered. I will focus in particular on matters related to genomic surveillance and data sharing, methodological advances for phylogenetic analyses, issues related to reproducibility, publication practices from the use of preprints to the availability of articles. I will discuss the importance of ORT for informing public health responses and ensuring trust from the public, and how ORT will be critical to better pandemic preparedness.



Three species, three labs, three experiments: testing the reproducibility of evolutionary-ecological studies on insect behaviour in a multi-laboratory setting

Mundinger C¹, Schulz N¹, Singh P², Janz S², Schurig M¹, Seidemann J³, **Kurtz J¹**, Müller C², Schielzeth H³, von Kortzfleisch V¹, Richter H¹

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MEETING ROOM 122+123, August 19, 2025, 2:00 PM - 4:00 PM

The reproducibility of studies involving insect species is an underexplored area in the broader discussion about poor reproducibility in science. Our study addresses this gap by conducting a systematic multi-laboratory investigation into the reproducibility of evolutionary-ecological studies on insect behaviour. We implemented a 3 x 3 experimental design, incorporating three study sites, and three independent experiments on three insect species from different orders: the turnip sawfly (*Athalia rosae*, Hymenoptera), the meadow grasshopper (*Pseudochorthippus parallelus*, Orthoptera) and the red flour beetle (*Tribolium castaneum*, Coleoptera). Using random-effect meta-analysis, we compared the consistency and accuracy of treatment effects on insect behavioural traits across replicate experiments. We successfully reproduced the overall statistical treatment effect in 83% of the replicate experiments, but overall effect size replication was

achieved in only 66% of the replicates. Thus, though demonstrating sufficient reproducibility in some measures, this study also provides first experimental evidence for cases of poor reproducibility in insect experiments. Our findings further show that reasons causing poor reproducibility established in rodent research also hold for other study organisms and research questions. We believe that a rethinking of current best practices is required to face reproducibility issues in insect studies, but also across disciplines. Specifically, we advocate for adopting open research practices and the implementation of methodological strategies that reduce bias and problems arising from over-standardization. With respect to the latter, the introduction of systematic variation through multi-laboratory or heterogenized designs may contribute to improved reproducibility in studies involving any living organisms.



Towards an open and responsible publication model

Radwan J¹

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MEETING ROOM 122+123, August 19, 2025, 2:00 PM - 4:00 PM

Publication in scientific journals relying on peer review to ensure reliability is a dominating model of scientific communication, but with an exponential increase in the number of articles published in the last decade, the model becomes dysfunctional, putting the credibility of science in threat. I will review evidence for this crisis, including an increasing share of papers produced in papermills that find their way even to highly indexed journals. The crisis reflects increasing pressure for journals to publish more articles, and difficulties editorial boards encounter in contracting good-quality reviews, a consequence of ever increasing number of article submissions. I will argue that the crisis is significantly associated with currently predominating open access publication model based on so-called article processing charges (APCs), which turn scientific publishing into billion-euros industry. I will illustrate the resulting conflict of quantity vs. quality with publicly known cases of resignations of editorial boards being under pressure to accept and an ever increasing number of articles. Another highly undesirable consequence of article

processing charges is a barrier they impose for scientists from the global south. This diagnosis suggests ways to solve the crisis. The solution should re-direct taxpayer money now spent on APSs to high-quality subscription-based publishers guaranteeing open access and high peer-review quality, for example, guaranteed by recognised societies like ESEB. In particular, publishers supported from public money should guarantee: (1) open access publication of a pre-negotiated number of articles per year, which will remove the incentive to publish more articles; (2) publicly known subscription rates to prevent unjustified revenues of publishers; (3) high quality peer reviews, which could be assessed by panels of experts. No restrictions should be imposed on publishers as to the affiliations of the authors in order to prevent geographic barriers to open publishing. The peer review crisis could be addressed by recognising reviewing process as an important scientific activity, whose quality should be assessed along with more traditional scientific achievements. This would need to involve open peer review, with likely additional positive effects on review quality.





ESEB2025
BARCELONA 17-22 AUGUST 2025

CONGRESS OF THE EUROPEAN SOCIETY FOR
EVOLUTIONARY BIOLOGY

S12 - Epigenetics and adaptation to global change: climate and biotic interactions



Developmental plasticities within and across generations in *Austrolebias* annual killifish

Van Dooren T, Olijerhoek J

POSTER SESSION 3 - **P03.044**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Predictions of global change responses need estimates of phenotypic plasticities. During development, embryos experience different interacting environmental effects, while their individual state is in part determined by their parents. Developmental plasticity to cues which parents or offspring experience can be active or passive. Either can be maladaptive, when that would decrease long-term fitness.

Annual killifish live in temporary ponds with egg banks of developing and diapausing embryos persisting through dry periods. Embryos of the Argentinian pearl killifish *Austrolebias bellottii* were subjected to three crossed environmental treatments, of which one was applied to their parents. Embryos were incubated in water that housed adult fish or not. They were incubated at four different temperatures. Parents experienced short- or long daylength regimes.

Effects on fitness components were estimated, namely stage-specific survival rates and rates of development. Multi-state models were used to predict distributions over developmental stages with age.

We found an overall acceleration of events with temperature, less pronounced for

mortalities. The data suggested that optimal temperatures for survival differ between stages. There were some effects of parental light regime and signals of adults in the water on embryo survival. Convergence to a diversified bet-hedging distribution of diapause stages occurred in each temperature. It is unclear whether this stability involved any active response. A deceleration of late development occurred in high temperatures when parents experienced the short day light regime. It is consistent with the idea that annual fish multigenerational reaction norms are adapted to a regular annual seasonal cycle. Overall, the data suggest that developmental plasticity is rather passive. Part of the variation in outcomes is due to mortality plasticity. Patterns which can be explained as adaptations combine adaptation to a regular seasonal cycle by a parental effect, and maybe diversified bet-hedging. However, this assumes adaptation to the past and it remains to be seen how climate change will change adaptiveness.



DNA methylation footprint of fisheries in genetically homogeneous marine metapopulations

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POSTER SESSION 3 - **P03.012**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Trait selective harvesting by fisheries can impose selective pressures that can induce measurable changes in fish life histories, ultimately affecting fisheries productivity and ecosystem functioning. While the genetic consequences of harvesting have been extensively studied, the extent to which variation on phenotypic traits reflects genomic evolution versus environmentally-induced plasticity remains unresolved. DNA methylation may mediate between these mechanisms, acting as a rapid, environmentally responsive regulatory process. In this study, we examined both, genomic variation and DNA methylation patterns in the marine fish *Xyrichtys novacula* across three geographical areas comprising two adjacent sites subject to contrasting fishing pressures; including a no-take Marine Protected Area (ntMPA) and an exploited fishery. A nested analysis design across the three spatial replicates, revealed strong gene flow and no evidence of genetic

structuring between sites. However, significant reductions in nucleotide diversity were observed in fisheries relative to ntMPAs, suggesting an increase in genetic diversity linked to protection. In contrast, DNA methylation differences revealed a more pronounced and consistent divergence across protection levels and geographical areas, suggesting an effect of fishing pressure on DNA methylation patterns. We identified numerous differentially methylated CpG sites, primarily located within coding regions, including both introns and exons. These sites were associated with genes involved in metabolic regulation and neural development, suggesting a potential role for DNA methylation in mediating phenotypic plasticity in response to fishing pressure. Our work represents one of the first lines of evidence that fisheries may exert evolutionary influence via DNA methylation modifications without altering the underlying the DNA sequence.



Epigenetic factors associated with diel vertical migration and diapause in the marine copepod *Calanus finmarchicus*.

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POSTER SESSION 3 - **P03.061**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

As ocean temperatures increase due to climate change, many temperate marine species are experiencing habitat range shifts to higher latitudes. One such species is *Calanus finmarchicus*, an abundant copepod in the North Atlantic Ocean, where it serves as a food source for higher trophic levels and plays a key role in biogeochemical carbon cycling by consuming phytoplankton and moving fixed carbon to the deep ocean. *C. finmarchicus* displays two migration behaviours crucial to its ecological role, i.e. diel vertical migration (DVM) and seasonal migration behaviour associated with developmental diapause. While DVM follows a circadian pattern, with copepods ascending to the surface to feed at night and descending to deeper water during the day to avoid predators, diapause occurs in late spring when copepods in their penultimate copepodite stage migrate to the deep sea where they remain inactive for 7-8 months. Photoperiod may play a significant role in regulating both DVM and the timing of diapause, implicating phenological shifts in seasonal cues as major potential disruptors of *Calanus* ecology with cascading effects on marine ecosystems. However, our understanding of the extent of phenotypic plasticity in their migration behaviours and the underpinning physiological and

molecular processes remains limited. Epigenetic mechanisms, such as DNA methylation, are important sources of molecular plasticity in response to environmental stimuli and affect gene expression and higher phenotypes. While the role of epigenetics in chronobiological processes has been explored in vertebrates and terrestrial invertebrates, it remains understudied in marine invertebrates. Here, I identify the role of DNA methylation in DVM and diapause in *Calanus finmarchicus* behaviour using samples of this species from temperate (Loch Etive, West Scotland) and sub-Arctic (Fram Strait, Greenland Sea, Svalbard) locations. Using Enzyme-Linked Immunosorbent Assay (ELISA) and rhythmic analysis, I tracked genome-wide methylation changes through the circadian and circannual cycles. I identified locus-specific methylation changes in circadian and diapause-associated genes by real-time quantitative PCR (qPCR). Preliminary data revealing rhythmic global DNA methylation changes suggest epigenetic involvement in endogenous control of migration behaviours in *Calanus*. This work offers new insights into the molecular basis of these crucial behaviours and lays the foundation for microcosm experiments that will enable mechanistic insight into these processes.



Epigenetic response of mountain hare (*Lepus timidus*) in different climate zones

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POSTER SESSION 3 - **P03.002**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The mountain hare (*Lepus timidus*) is a boreal mammal with a wide distribution across northern Europe, ranging across dramatically different climate zones, from mild winters in Ireland and strong winter conditions like in the Alps and Norway. Here, we investigated DNA methylation patterns in liver samples from individuals legally hunted in these three areas to investigate whether epigenetic responses varied among individuals. Pairwise population comparisons showed that differentially methylated regions were located in genes involved in pathways determining thermoregulation, pigmentation and developmental processes linked to body size. In addition, epigenetic distances between populations mirrored previously

published genetic distances for the same populations, and several differentially methylated genes were those reported to have fixed alleles. In both pairwise comparisons including Ireland (which do not universally moult to a white pelage in winter) pigmentation genes were hypomethylated. Comparing Alpine (smaller body size) and Norwegian (larger) hares, the latter had mostly hypomethylated genes in the pathway developmental process assumed to underlie the observed differences in body mass index. This indicated that in addition to genetic changes, epigenetic modifications may also contribute to the molecular basis of Bergmann's rule. Overall, our results suggest that epigenetic mechanisms may increase adaptability to rapid environmental changes.



Epigenomic profiles underlying induced defenses in a freshwater gastropod

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POSTER SESSION 3 - **P03.033**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Phenotypic plasticity describes the ability of a genotype to differentially express phenotypes in response to environmental cues. A well-studied instance of plasticity are the phenotypic changes triggered by cues indicating predation risk, also known as inducible defenses. One pivotal model for studying these defenses is the freshwater gastropod *Physella acuta*, where damage-released cues from conspecifics (i.e., alarm cues) are known to induce changes to their behavior, morphology and life-history that are beneficial in a predation context. Despite extensive research on these phenotypic changes, the underlying gene expression profiles remain largely unexplored. In this study, using *P. acuta*, we employed a split-clutch design to investigate risk-induced changes in gene accessibility. One half of full siblings was raised from hatching onwards in

a high-risk environment, i.e., under continuous exposure to conspecific alarm cues. The other half was raised in a low-risk water control environment. We sampled snails' behavior and morphology at sexual maturity, shortly before obtaining foot tissue for ATAC-sequencing. Differentially expressed open chromatin sections between risk treatments will then be characterized using our functional annotation of a recent *P. acuta* genome assembly. We will not only showcase treatment-dependent up- and downregulation of *P. acuta* genes but also correlate them to individual phenotypic changes. This research aims to elucidate epigenomic changes underlying phenotypic plasticity, thereby contributing to an improved understanding of the molecular mechanisms driving adaptive responses within single generations.



Evolutionary Trade-offs Between Intergenerational and Transgenerational Fitness Effects

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POSTER SESSION 3 - P03.045, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Intergenerational and transgenerational fitness effects can shape evolutionary processes. Theoretically, however, intergenerational and transgenerational effects can trade-off with each other with profound consequences for population dynamics. Here we show that beneficial intergenerational effects that increase offspring fitness can result in detrimental transgenerational effects that decrease great-grand offspring fitness. We used theoretical and experimental techniques to investigate transgenerational fitness effects of larval starvation in *Caenorhabditis elegans*. We show that individuals who undergo larval starvation exhibit large decreases in multiple fitness estimates.

However, regardless of offspring environment, their immediate descendants gain fitness benefits whilst great-grandchildren suffer fitness costs. Through simulating boom-and-bust population dynamics, we find an adaptive evolutionary strategy where beneficial intergenerational effects trade-off with detrimental transgenerational effects to increase fitness of a genotype across many generations. Our findings suggest that such multigenerational trade-offs will play an important role in evolution and challenge the view that transgenerational effects are necessarily adaptive.



Evolved transcriptional responses and their regulation after long-term adaptation of *Bemisia tabaci* to a marginally-suitable host

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POSTER SESSION 3 - **P03.089**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Although invasive generalist insect herbivores can migrate and rapidly adapt to a broad range of suitable host plants, they can face significant difficulties when accidentally migrating to novel and marginally-suitable hosts. What happens, at both the genome-wide regulatory and transcriptional levels, if these marginally-suitable hosts must be used for multiple generations before migration to a suitable host can take place, largely remains unknown. Moreover, if adaptation to the new marginally-suitable hosts and transcriptomic changes do occur, how are they regulated? Does it involve changes in epigenetic patterning and processing (such as alterations in DNA methylation) and/or sequence changes in cis- or trans-regulatory elements in the genome (such as enhancers and promoters)? Using as a model the invasive and generalist whitefly species, *Bemisia tabaci*, we established a multi-generational experimental system that allowed us to explore the evolved differences between two populations that were subjected to cotton (a suitable host) and habanero-pepper (a marginally-suitable host) on which the initial survival is 5%.

First, we conducted reciprocal host performance assays under both optimal (30°C) and mildly stressful (24°C) temperature conditions to assess the adaptive changes in the two populations. We found that the habanero-adapted population exhibited a substantial increase in survival on habanero pepper (exceeding 60%) but did not reach the survival rates observed on the original cotton host (80–90%). Furthermore, this population displayed a trade-off, exhibiting reduced performance on cotton relative to the non-adapted population, and an antagonistic interaction with temperature stress at 24°C. Transcriptomic profiling revealed that adaptation to habanero pepper was associated with two major molecular changes: (1) an upregulation of genes involved in cuticle

structural component synthesis and (2) a downregulation of genes encoding cysteine-type peptidases, particularly cathepsin B proteins, which are implicated in the activation of plant defense responses.

Next, we analyzed the evolution of 5-methylcytosine (5mC) DNA methylation and single nucleotide polymorphisms (SNPs) in promoter regions after 34 generations. Our findings revealed only slight divergence in 5mC methylation levels between populations reared on suitable versus marginally-suitable hosts. These methylation differences were not directly correlated with changes in gene expression levels. Instead, we observed a strong association between 5mC methylation patterns, gene function, and expression variability. Housekeeping genes exhibited hypermethylation and lower expression variability, whereas environmentally responsive genes were hypomethylated and displayed higher expression variability. Additionally, SNP analysis in promoter regions suggested selection for specific alleles that may contribute to adaptive processes. These findings indicate that both epigenetic modifications and genetic variation in regulatory elements play a role in the multi-generational adaptation of invasive generalist herbivores to marginally-suitable hosts.

In conclusion, our study provides novel insights into the interplay between epigenetic regulation and genetic selection during host adaptation in invasive insect herbivores. The observed shifts in gene expression, 5mC methylation, and promoter-region polymorphisms highlight the complex mechanisms underlying long-term adaptation to suboptimal host plants. These results contribute to a broader understanding of how invasive generalists persist and evolve in novel environments, with implications for pest management and ecological resilience.



Experimental evidence for short term directional selection of epigenetic trait variation

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POSTER SESSION 3 - P03.022, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Evolution by natural selection can occur when organisms harbor genetically inherited phenotypic variation, and phenotypic variants have differential fitness. Stable transgenerational epigenetic variation also exists for fitness-related traits and theory predicts that selection can act on this variation alone without a contribution of genetic variation. In this talk, I will present how we artificially selected for divergent biomass, rosette size, flowering time and height at first silique in experimental

Arabidopsis thaliana populations harboring DNA methylation polymorphism in an identical genetic background. We found significant epi-allele frequency changes in response to selection. Our results show how selection rapidly changed population trait values and their epigenetic basis, over one generation of selection. Our results imply the role of the transgenerational epigenetic variation of populations as an additional source of short-term adaptive potential.



Exploring the link between epigenetics and deleterious mutations

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POSTER SESSION 3 - P03.085, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Quantifying the functional effects of genetic mutations is a central challenge in evolutionary biology. Beyond amino acid changes, mutations affecting gene regulation can also have fitness consequences. Here, we argue that, due to their pivotal role in gene regulation, epigenetic mechanisms likely modulate the expression and persistence of deleterious mutations. While epigenetics has been linked to inbreeding depression in model organisms, this connection remains largely unexplored in wild populations. Drawing upon evidence from humans and model organisms, we will highlight four potential interactions between epigenetics and deleterious mutations. We hypothesise that

epigenetic mechanisms could (i) be disrupted by deleterious mutations in non-coding regions; (ii) buffer the expression of deleterious mutations; and (iii) contribute to the repair and purging of deleterious mutations. Harnessing next-generation sequencing and bioinformatics, empirical testing of these hypotheses is both feasible and necessary. Unravelling the interplay between epigenetics and deleterious mutations is essential for assessing the fitness effects of genetic variants, which has significant implications for ecological and evolutionary processes, as well as for biological conservation, especially in a rapidly changing world.



Genetic and epigenetic responses of a keystone forest tree species to global climate change revealed through the integration of population-scale pangenomics and multi-omics analyses

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POSTER SESSION 3 - **P03.054**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Global climate change poses a significant threat to ecosystem stability. Forests, as a key component of terrestrial ecosystems, play a vital role in the global carbon cycle. Unlike animals, plants are sessile and unable to escape unfavorable abiotic stresses through migration. Instead, they have evolved intricate mechanisms to adapt to environmental changes. Among these, rapid epigenetic modifications, along with changes in gene regulation and expression, represent critical and immediate responses to environmental stresses and resistance. However, our understanding of the relative contributions of genetic and epigenetic variations to both short-term abiotic stress responses and long-term evolutionary adaptations of populations to diverse climates remains limited. Here, we aim to integrate pangenomics, population genomics, and stress-induced multi-omics analyses to gain novel insights into how structural variations and transposon dynamics drive stress responses and adaptation to climate change, in conjunction with gene regulatory modulation and epigenetic modifications.

Materials and Methods: This study focuses on *Populus lasiocarpa*, a dominant and endemic alpine forest tree species with a ring-like geographic distribution surrounding the Sichuan Basin in central Asia. We integrated a 20 haplotype-scale pan-genome assembly, 200 population-scale whole-genome resequencing datasets, and various abiotic stress-induced (including heat, cold, drought, and waterlogging) RNA-seq, ATAC-seq, and Bisulfite-seq datasets to comprehensively explore how genetic variation interacts with gene regulation and epigenetic modifications. This approach aims to uncover the mechanisms underlying immediate stress responses and long-term evolutionary adaptations in natural populations of this keystone forest tree species.

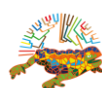
Results: First, by combining HiFi long-read sequencing with Hi-C scaffolding technologies, we assembled 20 haplotype-scale genomes for ten representative individuals sampled throughout the natural distribution of the species. By integrating high-depth whole-genome resequencing datasets from 200 individuals across 22 natural populations, we constructed the pangenome of *P. lasiocarpa*

and identified structural variations (SVs) and transposon polymorphisms (TIPs) at the population scale across the entire genome.

Second, as these populations primarily group into two genetic clusters (western and eastern), we conducted greenhouse experiments to treat seedlings under various abiotic stress conditions. This allowed us to examine gene expression, chromatin accessibility, and methylation variations across both genetic clusters and stress treatments. We identified differentially expressed genes (DEGs), differential accessible chromatin regions (dACRs), and differentially methylated regions (DMRs) in response to abiotic stresses. These regions were further associated with pangenome characteristics, as well as dynamic SVs and transposable elements (TEs) across populations, to explore the interaction between genetic and epigenetic mechanisms in regulating stress responses and adaptation.

Lastly, we investigated the evolutionary forces acting on genomic variations and epigenetic modifications. We performed a distribution of fitness effects (DFE) analysis to assess the strength of purifying selection, as well as genotype-environment association (GEA) analyses to identify potential adaptive variants driving environmental adaptation. From these analyses, we selected candidate adaptive SVs and TIPs for experimental validation.

Conclusion: In summary, our study integrates pan-genomic and epigenetic approaches to comprehensively elucidate the molecular mechanisms underlying the responses to abiotic stress in *P. lasiocarpa*. This work provides valuable insights into both the immediate responses to environmental stresses and the long-term climate adaptation of these keystone species. Furthermore, we identified a set of adaptive variants, including both SVs and TIPs, that are strongly associated with gene regulation and stress responses, which offer a valuable genomic resource for future tree breeding programs aimed at enhancing adaptation to changing environmental conditions.



Identification of Fish Epigenetic Biomarkers of Temperature Response and parasite resistance shaped by Early Rearing Conditions

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*POSTER SESSION 3 - **P03.001**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM*

Environmental variation can trigger epigenetic changes that regulate gene expression without altering the underlying genetic information. Understanding this environmentally induced, non-genetic inheritance may provide valuable insights into phenotypic plasticity and adaptive evolution. In the context of climate change, rising temperatures are expected to influence disease dynamics, potentially altering pathogen resistance patterns in aquatic species. In this study, we aim to identify epigenetic markers associated with early rearing conditions, focusing on

temperature response and parasite resistance, using Oxford Nanopore sequencing. We analyze methylation differences between Atlantic salmon families reared under varying temperature conditions and when exposed to two pathogens: ISAV (Infectious Salmon Anemia Virus) and CMS (Cardiomyopathy Syndrome). Our findings offer new insights into how epigenetic mechanisms shape environmental responses in non-model species.



Temperature dependent DNA methylation in the Swedish sand lizard (*Lacerta agilis*)

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POSTER SESSION 3 - **P03.059**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Sand lizards (*Lacerta agilis*) are found across Europe, even stretching as far north to extremely cold environments such as in Sweden. Being highly adapted to such extreme temperatures makes them highly susceptible to climate change. Hence, we have been investigating how temperature may impact epigenetic mechanisms during egg incubation. Using an experimentally isolated, outbred, island population in southern Sweden we conducted temperature incubation experiments. Followed by reduced representation bisulfite sequencing of hatchling tail tissue to assess the role of temperature-dependent DNA methylation in early life stages and its thermolability. After identifying differentially methylated sites will determine their

association to promoters, gene bodies, intergenic regions and repeats, and follow up with gene ontology analysis to infer any impacts on gene function. Previous work on this dataset found thermolability of sex and sex-specific telomere dynamics, and using this additional existing data we aim to determine any epigenetic contributions to these dynamics. This study will greatly improve our understanding of epigenetic regulation in reptiles in general but also give us key insights into the impacts of climate change on the Swedish sand lizard in the near future. Our results, will help to inform the conservation of the species in Sweden, which will have to adapt to new seasonal temperatures as a result of climate change.



The epigenetic dimension of effects of climate change on alpine plants

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POSTER SESSION 3 - P03.015, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Climate change and extreme episodes of environmental stresses, notably heatwaves and droughts, have significant impacts on ecosystems and biodiversity. These stressors drive shifts in species distributions and demand rapid adaptation to ensure survival. The adaptive capacity of populations depends on heritable phenotypic variation that can be filtered by natural selection leading to locally adapted populations. However, it is questionable whether such adaptations can occur fast enough to keep pace with rapidly changing environments. Alongside long-term adaptive responses, shorter-term mechanisms underlying phenotypic plasticity can also contribute to successful persistence under environmental challenges. Among these mechanisms, the potential role of epigenetic variation—such as DNA methylation and its influence on gene expression—in acclimation and adaptation to climate change has been largely overlooked. Using transplantation experiments along altitudinal gradients, we investigate in natural habitats the molecular responses to climate warming of four representative unrelated high-alpine plant species, including *Luzula alpino-pilosa*, *Phyteuma hemisphaericum*, *Sibbaldia procumbens* and *Veronica alpina*.

To simulate the effects of climate warming in natural settings, we implemented a long-term in situ transplantation experiment, contrasting gene expression and DNA methylation across relocated plants from their native high-elevation habitats to lower elevation sites and high elevation control sites. This approach resulted in a consistent and ecologically relevant temperature increase of approximately 2.5°C during the growing seasons of the past four years, when compared to control plots relocated within the original elevation range. Importantly, this experimental design allowed us to study plant

responses under natural conditions while measuring survival and growth of the transplanted individuals.

Analyzing high-coverage short-read sequencing of enzymatically converted DNA libraries against newly assembled reference genomes, we detected significant variation in global DNA methylation levels induced by the temperature difference. In particular, under long-term temperature stress replicates exhibited increased variation in DNA methylation, compared to controls. Thousands of differentially methylated regions across the three types of sequence contexts of methylation in response to the warming treatment have also been recorded, most affecting regions outside of CDS. These changes suggest a potential epigenetic mechanism underlying plant acclimation to climate stress mainly driven from stochastic effects. Accompanying mRNA sequencing revealed a complex relationship between differential DNA methylation and changes in gene expression, underscoring the intricate regulation of plant stress responses at the molecular level. Functional enrichment analyses of both differentially expressed genes and differentially methylated regions point toward relevant stress-responsive functions and suggest a partial acclimation to the new, warmer conditions.

The insights gained are currently integrated with detailed ecological information from complementary projects to enhance our understanding of the extent and limits of biological responses to climate change in natural settings. Our findings grant a fuller appreciation of the spectrum of molecular and ecological implications of climate change and are expected to result in improved conservation strategies for alpine plants facing climate change.



Unveiling the genetic basis of floral seasonal phenotypic plasticity in the Moricandia genus

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POSTER SESSION 3 - P03.078, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Phenotypic plasticity enables organisms to modify their phenotype in direct response to environmental changes. This crucial property may become essential in the era of climate change, as it may allow individuals to rapidly change their morphology, behavior, and physiology to endure increasingly stressful conditions. Central to this adaptability are shifts in gene expression, which environmental cues directly influence. Transcriptomics helps identify the genes and regulatory pathways activated in response to temporal or spatial environmental variations, providing insights into ecological acclimatization and short-term adaptation. *Moricandia arvensis*, a Mediterranean mustard, exemplifies the strength of phenotypic plasticity. This plant thrives in arid environments by adjusting its photosynthetic traits to withstand extreme summer conditions. Additionally, it demonstrates a striking floral polyphenism, producing two disparate flower types depending on the season. In the *Moricandia* genus, different species exhibit varying levels of plasticity in several floral traits, ranging from no plasticity

to extreme plasticity, such as the floral polyphenism seen in *M. arvensis*.

Materials and methods: To comprehensively understand the genetic basis of floral seasonal phenotypic plasticity in the *Moricandia* genus, we undertook a comparative transcriptomics analysis across various species of *Moricandia* under experimental spring and summer conditions.

Results: Our findings indicate that the intraindividual phenotypic shift between these two seasons is associated with crucial transcriptional changes in flowers. This analysis highlights shared gene expression patterns and species-specific responses, ultimately shedding light on the genes that contribute to seasonal phenotypic plasticity. Each species demonstrates unique transcriptomic responses while also exhibiting some common molecular signatures.

Conclusion: This research contributes to our understanding of phenotypic plasticity within the *Moricandia* genus and serves as a valuable model for investigating the evolution of phenotypic plasticity.



A Hardy–Weinberg Equilibrium–Based Neutral Model for Plant DNA Cytosine Methylation Variation

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MEETING ROOM 120+121, August 22, 2025, 2:00 PM - 3:00 PM

Although some plant epigenetic marks are environmentally responsive, molecular studies have shown that other CG methylation variants can be stably transmitted across generations. Understanding how stably inherited CG methylation variation is maintained within populations is vital not only for elucidating the evolutionary basis of stable epigenetic variants but also for forecasting how environmentally responsive marks might influence population-level methylation patterns. However, our insight into the evolutionary significance of plant methylation variation has been limited by the absence of a neutral null model. To address this gap, we developed a Hardy–Weinberg equilibrium (HWE)–based null model and used it to test how observed methylation-state frequencies deviate from neutral expectations.

In classical HWE, allele and genotype frequencies remain constant in a large, randomly mating population free of selection, mutation, migration, and drift. We adapted this framework to methylation epialleles by modeling the distribution of methylated (M) and unmethylated (U) genotypes under neutrality. Any excess or deficit of M/M, M/U, or U/U epigenotypes thus flags non-random processes—selection, environment-induced change, or drift.

We further incorporated two methylation-specific features observed in *Arabidopsis thaliana*: (1) a slightly higher rate of loss of methylation ($M \rightarrow U$) than gain ($U \rightarrow M$), and (2) hybrid-specific remodelling (transchromosomal methylation/demethylation, TCM/TCdM), which can produce additive or non-additive patterns. We then ran finite-population simulations, including scenarios of selfing (reflecting *A. thaliana*'s mating system) and

demographic expansion, to generate methylation site frequency spectra (mSFS) under (a) neutrality, (b) neutrality plus selfing, and (c) neutrality plus population expansion. Neutral models incorporate biased epimutation rates and methylation remodelling.

Our results show that TCM/TCdM exerts the strongest influence on methylation-state variation; biased epimutation alone drives fixation toward U/U, but remodeling maintains polymorphism in a population. Selfing reduces heterozygous (M/U) states, with overall diversity shaped by the balance between selfing and TCM/TCdM. Population expansion reduces variance and reduces the time to fixation. It results in the characteristic U-shaped mSFS, which indicates an excess of rare methylation states, that has been observed in *A. thaliana* populations.

This is the first neutral, population-genetics–based model specifically for plant methylation. By integrating known molecular mechanisms in *A. thaliana* with classical evolutionary theory, we demonstrate that epigenetic remodeling is the principal force maintaining variation. In contrast, biased epimutation, selfing, and demography contribute to an excess of rare methylated and unmethylated states, with unmethylated states being more common due to the directional bias in epimutation rates. Our model lays the groundwork for future tests of non-neutral influences such as selection and migration on plant methylation variation. It is also useful to examine how the combined influence of environmentally inducible methylation states and transgenerationally stable methylation variants shapes epigenetic variation within populations.



DNA methylation: a way for fast adaptation in clonal species? An example with the plant species *Fragaria vesca* and adaptation to altitude.

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MEETING ROOM 120+121, August 22, 2025, 10:30 AM - 12:30 PM

Given their sessile nature, plants need to constantly respond to changing environments, i.e. show phenotypic plasticity, essential in the context of climate change. DNA methylation and other epigenetic modifications may enable rapid adaptation to environmental changes by generating locally adapted phenotypes. However, the epigenome is not usually considered a mechanism for long-term adaptation that can respond to selection, due to the potentially limited inheritance of epigenetic marks, especially during sexual reproduction, where epigenetic resetting occurs during meiosis. However, clonal propagation in plant bypasses this resetting process. In clonal species, epigenetic variation (or epi-allele) might serve as a substrate for selection, and could be particularly important for long-term adaptation, especially if clonal propagation is associated with reduced genetic diversity. This assumption remains, however, to be tested in natural populations.

Among all epigenetic marks, DNA methylation has received the most attention in evolutionary studies. In plants, DNA methylation occurs in three sequence contexts: CG, CHG and CHH. DNA methylation variation arises at a considerably higher rate than genetic mutations in response to genetic, environmental and stochastic factors. In all sequence contexts, epi-allele plays a pivotal role in repressing the mobilisation of transposable elements, but only CG and CHH methylation appear to be connected to gene expression. Thus, epi-alleles, as genetic mutations, meet all the criteria required for natural selection to act in clonal species: they can vary within and between natural populations, be inherited across generations, and influence individual fitness.

Using population genomics approaches, we investigated whether epi-alleles are subject to

natural selection in the clonal plant *Fragaria vesca*. This species has a wide natural distribution and can inhabit a range of altitudinal environments. In this study, we focused on from three low- and four high-altitude Italian populations which a substantial amount of publicly genomic, epigenomic, and transcriptomic data existing for clones cultivated under common garden conditions. The environmental effects on epi-alleles were minimised by the cultivation in common garden, allowing us to focus on inherited epi-alleles that may have been selected in response to altitude gradient in ancestral populations.

First, we demonstrated that CG and CHG patterns clustered individuals by ancestral populations and altitude of origin, and these pattern were only partially explained by genetic variations. Moreover, the linkage disequilibrium between epi-alleles appeared to be independent to genetic variations. These two observations suggested that epi-alleles, including them under natural selection, are partially independent of the genetic variations. Employing the classical fixation index, we identified 136 heritable candidate regions under altitudinal selection. These loci overlapped with genes involved in the O-methyltransferase activity. However, only one of them overlapped with epi-alleles associated with altitude by epigenome-wide association (EWA) approach and none of them was associated with the phenotypic traits measured. Finally, we distinguished epi-alleles under selection that were regulated by genomic variation, revealing that most of the epi-alleles under selection in the two altitude groups were non-genetically determined.

These findings suggest that epi-allele could be an essential way of adaption in clonal species, potentially compensating for their low genetic variation.



Epigenetic differences from floater to territory holding male Namibian cheetahs (*Acinonyx jubatus*)

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MEETING ROOM 120+121, August 22, 2025, 10:30 AM - 12:30 PM

Within a lifespan, one genotype can bring about several phenotypes. Epigenetic mechanisms are strong candidates of molecular regulation for more rapid phenotypic changes. While changes within the DNA sequence are rare and slowly accumulate over generations, DNA methylation can change flexibly, regulate gene expression, and have the potential to increase adaptability of species facing environmental alterations. In free-ranging cheetahs, adult males can change their spatial tactics from roaming over large distances (“floater male”) to occupying a territory (“territorial male”). In consequence of such an event, males decrease their movement activity, undergo physical changes by increasing body mass, and show reduced anxious behaviour towards humans. With our study, we aimed to identify whether the change in spatial tactic changes DNA methylation patterns, whether genes and physiological pathways are affected associated with the observed phenotypic changes, and whether we can select epigenetic biomarkers that predict spatial

tactic of male cheetahs. Blood of ten free-ranging Namibian male cheetahs was taken twice, first when they were floaters and second after becoming territory holders. DNA from peripheral blood mononuclear cells (PBMCs) was enriched for methylated DNA by capture via methyl-binding-domain protein 2 (MBD2) and subsequent high-throughput sequencing (MBD-Seq). Using the methylation patterns from each individual's floater period as baseline, we detected 442 hypermethylated and 20 hypomethylated genes after cheetahs had become territorial. Differentially methylated genes were enriched in 23 pathways including development of anatomic structure, system, organ, nervous system, regulation of cell communication and signaling. The observed pathways associated with the observed behavioral, physiological and morphological changes, and indicate that DNA methylation might be important in the molecular regulation of shifting between spatial tactics in male cheetahs.



Epigenetic potential and range expansion in the house sparrow

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MEETING ROOM 120+121, August 22, 2025, 2:00 PM - 3:00 PM

House sparrows (*Passer domesticus*) are among the most common and broadly distributed vertebrates in the world. Although some of that success is due to the anthropophilic nature of the species, house sparrows also appear to have been successful colonizers because of their exceptional disposition to be plastic. In less than 200 years, sparrows have come to occupy all continents but Antarctica, and in the areas where they now reside, populations exhibit phenotypic patterns that suggest they have adapted to prevailing conditions much faster than would be expected from selection on standing genetic variation. In this presentation, we will summarize what we have revealed about the role of epigenetic potential in range expansions for this species, in particular how the CpG content of gene regulatory regions has enabled individuals to invade and thrive in new areas. We hypothesize that individuals that have high CpG density in gene promoters are better able to regulate expression of key genes. We think that this regulatory nimbleness protects them from novel and

threatening conditions common to new areas. In particular, we have been investigating how epigenetic regulation in the form of DNA methylation has helped sparrows cope with the diets they consume in new areas. A growing dataset from a variety of studies indicates that birds with the highest epigenetic potential are well-protected against gut pathogen infections (e.g., *Salmonella enterica*). Additional data indicate that these phenotypes manifest via DNA methylation of CpG sites in the promoters of the Toll-like receptor (TLRs) genes, sensors integral to surveillance for diverse pathogens. Indeed, TLRs are the first points of contact between pathogens and hosts, and moreover they determine the form and magnitude of host immune responses to microbes. Epigenetic potential in these genes in particular is probably adaptive and plastic because it enables individuals to mobilize the most promising immune response to an unknown threat in a manner that effectively balances the risks of mortality and morbidity with the energetic and nutritive costs of defense.



Epimutations in *Neurospora crassa*

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MEETING ROOM 120+121, August 22, 2025, 10:30 AM - 12:30 PM

While traditionally DNA sequence was considered to be the sole carrier of hereditary information, studies in recent years have revealed that epigenetic changes can contribute to heredity as well. While different definitions of epigenetics exist, we define epigenetic changes as changes in chromatin modifications, such as DNA methylation or histone modifications, that can be inherited.

If epigenetic changes can be transmitted across generations and it can contribute to phenotypic variation, as they potentially can as they can affect transcription, they can also be under selection. Theoretical studies have revealed that the properties of epigenetic variation will determine how epigenetic changes influence adaptation. To understand whether epigenetic changes are important for evolution, we must understand their properties.

We investigated the properties of epimutations in the filamentous fungus *Neurospora crassa*. To determine the rates spontaneous epimutations, that is spontaneous changes in DNA methylation and histone modifications, and to

understand where in the genome epimutation happen, we performed a mutation accumulation experiment, and sequenced MA lines across the pedigree. We observed that in *N. crassa* spontaneous DNA methylation changes do happen, but these are reliably transmitted across mitoses only in centromeric and pericentromeric regions. Rates of spontaneous DNA methylation changes are orders of magnitude faster than genetic mutation rates. With gain of methylation happening around $7-8 \times 10^{-6}$ per cytosine, and loss of methylation happening around $4-5 \times 10^{-4}$ per cytosine. Thus losses are around 50 times more common than gains. Ephemeral changes in DNA methylation do happen in euchromatic regions, but these are either not transmitted or their reversion rate is too fast to observe their accumulation in the MA pedigree.

Our results contrast to what has been observed in plants, and suggest that there are different pathways in *N. crassa* by which epimutations originate. We are currently testing this hypothesis using different mutants.



Maternal RNA variability as an adaptive feature of environmental responsiveness and heritable diversification

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MEETING ROOM 120+121, August 22, 2025, 10:30 AM - 12:30 PM

Heritable maternal RNAs are widely recognized as essential and highly conserved developmental cues. Yet beyond this canonical role, they may also function as sensitive indicators of environmental change and transmit adaptively meaningful information to the next generation. In organisms with high fecundity such as zebrafish, changes to the maternal RNA load may represent an adaptive bet-hedging strategy in the face of fluctuating environments. In particular, we are interested in change in maternal RNA variability, and investigate its role as signal rather than noise.

We thus currently investigate how heat stress influences the composition and variability of maternally deposited RNAs, and whether such changes are heritable across generations. In a multigenerational zebrafish experiment, fish were exposed to elevated temperatures for two weeks and mated at three post-stress timepoints (T1–T3). Unfertilized oocytes (up to 25 per mother) were collected individually, and subjected to RNA sequencing. They represent a transcriptionally and translationally stable stage, enabling high-resolution analysis of expression variability with minimal intrinsic noise.

The approach allows us to resolve maternal RNA variation at multiple levels—among eggs, within clutches, between mothers, and across treatments. Preliminary analyses reveal an increase in maternal RNA variability following heat exposure as well as an average increase in transcripts associated with the heat shock response. Principal component analysis and interquartile range metrics highlight numerous RNAs exhibiting treatment-specific variance shifts. Notably, gene expression patterns also correlate with maternal identity, suggesting an interaction between genetic background and environmental inputs in shaping variability.

These findings support the view that maternal RNA expression noise is not merely stochastic, but a potentially selectable trait modulated by stress. By characterizing its molecular drivers, functional identities, and transgenerational persistence, our work reframes gene expression variability as a critical axis of developmental and evolutionary plasticity. Together, these insights bridge developmental biology, epigenetics, and evolutionary ecology, and shed light on how organisms buffer and transmit information about environmental change through maternal contributions.



The role of epigenetics in local adaptation in sessile oak trees: a population epigenetic association study

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MEETING ROOM 120+121, August 22, 2025, 2:00 PM - 3:00 PM

Rapid climate change is forcing species to either adapt very quickly, shift their geographic distributions, or face local extinction. This challenge is especially acute for long-lived, sessile organisms such as trees, which cannot migrate easily and whose extended generation times limit the pace of evolutionary response through genetic variation alone. Epigenetic mechanisms, including DNA methylation, have been proposed as a means by which organisms may respond more quickly to environmental shifts. In this study, we examine the role of DNA methylation in local adaptation across populations of sessile oak (*Quercus petraea*).

We analysed DNA methylation patterns in 189 individuals from 22 populations spanning a latitudinal gradient from Southern France to Northern Germany. All individuals were grown in a common garden experiment in Sillegny, France, which controlled for environmental variability and allowed us to isolate heritable components of epigenetic variation. Methylation data were generated using a targeted sequence capture approach focusing on regions known to be epigenetically variable, and targeting specific candidate genes (Lesur et al., 2024). Methylation data was then imputed in all three contexts for using NIPALS as tested in Duplan et al. 2025.

Exploratory data analysis revealed low variability and differentiation in the data. The global F_{st} for the SNP data was 0.001, and the global P_{st} of the methylation data was 0.008 in all three contexts. A PLS-DA across contexts by population also reflected the low variability as the first two axes only explained <6% of the variation. Overall, we observed low levels of

DNA methylation variation across the genome, with fewer than 5% of SMPs exceeding 0.05 inter-individual variation in any context (CG, CHG, CHH). For downstream analyses, we retained only the most variable 5% of SMPs per context.

Multi-omic association analyses were conducted using a multi-locus mixed model (MLMM) framework that incorporated phenotypic, climatic, single nucleotide polymorphism (SNP), and methylation (SMP) data. Despite the low overall variation, retained SMPs exhibited significant associations with four different phenotypic traits and 26 different climatic variables. Additionally, 40%, 49%, and 20% of tested SMPs in CG, CHG, and CHH contexts, respectively, were significantly associated with SNPs under the MLMM. There is however a significant amount of spatial autocorrelation in the associated SMPs which may explain the high levels of association, this is expected as nearby methylation marks are known to experience co-methylation.

Our findings indicate that while global DNA methylation variation is limited in our *Q. petraea* populations, specific methylation markers are associated with phenotypic traits and environmental gradients, pointing to a potential—but constrained—role for epigenetic variation in local adaptation in this species. Recognising this limited yet meaningful contribution is critical for understanding the mechanisms by which long-lived species may respond to rapid climate change, especially during the early stages of adaptation when genetic responses may be too slow.



Transgenerational transmission of epigenetic responses as a second driver of adaptive evolution

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MEETING ROOM 120+121, August 22, 2025, 2:00 PM - 3:00 PM

Epigenetic molecular marking can be a mechanism for adaptation within generations, but also across generations. Transgenerational transmission of epigenetic responses is increasingly documented. I will first review how theoretical modelling has shown that when environmental change is too rapid for efficient genetic evolutionary tracking, this transgenerational transmission of epigenetic responses is expected to evolve. I will then argue that in the context of its ability to facilitate transgenerational change, epigenetic response is actually a subset of a wider category of organismal responses to ecological pressures. Subsequently, based on logical derivation and the Price equation, I will argue that organismal responsiveness, or plasticity in the broad sense, can be beneficially seen as a form of selection: as the non-random realization out of a set of options. When seen as a form of selection, broad-sense plasticity can then be seen as an alternative to natural selection for the adaptation of populations. With a reversed causality, here organisms are not selective targets, but selective agents. Following the logic of the breeder's equation (where evolutionary response $\approx$ selection pressure * degree of heredity), this alternative form of selection can thereby cause an evolutionary change in heritable traits when it is combined with non-genetic forms of heredity (as is sometimes the case for epigenetic responses but also for other organismal

responses). This combination of organismal plasticity (or response) and non-genetic inheritance hence provides a second driver of adaptive evolution. It could be summarised as “the selective production of heritable traits”. This can be contrasted with the classical driver of adaptive evolution, involving natural selection, or “the selective reproduction of heritable traits”. However, this additional second driver is only a driver of adaptive evolution if the organismal responses are adaptive. I will argue that this is not a given, but it is expected, since natural selection should act against maladaptive organismal responses. In this talk I therefore draw a parallel between the topic of the symposium, and the same issues for other forms of “selection” engaged in by organisms, such as trial-and-error learning, selective behavioural copying (cf. cultural evolution), partner choice (cf. social evolution), or habitat choice (cf. evolution of the extended phenotype). Epigenetic transmission is therefore a prime example of a wider discussion about the processes driving adaptive evolution. In the context of global change and evolutionary prediction, recognising the full spectrum of pathways towards organismal and evolutionary adaptation is perhaps more important than ever. (This talk is a partial summary of an Open Access book on adaptive evolution soon to appear at Oxford University Press.)





ESEB2025
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CONGRESS OF THE EUROPEAN SOCIETY FOR
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S13 - Evolution after whole genome duplication: transformative advances and new directions



A tektin gene family analysis of chromosome-level annelid genomes identifies an ancestral cluster of three tektin35B genes for one of the two major clades of Polynoidae/scaleworms

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POSTER SESSION 1 - **P01.141**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Tektin proteins form structural components within microtubules particularly in cilia and flagella. They play primarily involved in the organization and stability of axonemal structures, and are essential for cell motility and are broadly present across metazoans. Given their functional importance, Tektins could be informative to gain insights into the emergence of novel cilia specializations within spiralian, a superclade of over 14 animal phyla including mollusks (e.g., snails) and annelids (e.g., earthworms). Previous work revealed that the ancestral condition of the tektin gene complement for spiralian genomes was five tektin genes: Three (tektin-2, -1, and -4) are of metazoan and bilaterian origin, while two, tektin-35A and -35B are spiralian specific ones that originated from a duplication of the bilaterian tektin-35 gene at the base of all spiralian. Marine annelids (polychaetes), among the most diverse spiralian groups represented by close to 100 families, most of which possess free-swimming larval stages that are propelled by motile cilia organized in ciliary bands or trochs.

Materials and methods: To gain further insight into the evolution of the tektin

complement within annelids, we analyzed over 20 chromosome level annelid genomes focusing on the larger subclades of Errantia and Sedentaria using reiterative BLASTX searches and subsequent phylogenetic analyses.

Results: Intriguingly, we found that tektin-1, -2 and -4 remained single copy genes in both subclades, while the two spiralian-specific tektins-35A and -35B were frequently duplicated. Of particular interest is the discovery of an ancestral cluster of three tektin-35B genes, and two duplicated tektin-35A genes in one of the major subclades of the scaleworms/Polynoidae, with similar duplications in the derived vent associated scaleworm *Branchipolynoe longqiensis*. In contrast, the single representative for the most basal polynoid clade *Lepidonotus clava* exhibits a more ancestral tektin gene complement of six with a potentially independent 35B duplication. **Conclusions:** This study identifies distinct molecular synapomorphies in regard to the tektin complement, shows the potential of this approach to identify unique cilia specializations within subclades of annelids, and to link these to phenotypes of ciliated larvae in the future.



Asymmetric genome merging leads to gene expression novelty through nucleo-cytoplasmic disruptions and transcriptomic shock in *Chlamydomonas* triploids.

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POSTER SESSION 1 - P01.154, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

- Genome merging is a common phenomenon causing a wide range of consequences on phenotype, adaptation, and gene expression, yet its broader implications are not well understood. Two consequences of genome merging on gene expression remain particularly poorly understood: dosage effects and evolution of expression.

- We employed *Chlamydomonas reinhardtii* as a model to investigate the effects of asymmetric genome merging by crossing a diploid with a haploid strain to create a novel triploid line. Five independent clonal lineages derived from this triploid line were evolved for 425 asexual generations in a laboratory natural selection experiment.

- Utilizing fitness assays, flow cytometry, and RNA-Seq, we assessed the immediate consequences of genome merging and

subsequent evolution. Our findings reveal substantial alterations in genome size, gene expression, protein homeostasis, and cytonuclear stoichiometry. Gene expression exhibited expression-level dominance and transgressivity (i.e. expression level higher or lower than either parent). Ongoing expression-level dominance and a pattern of 'functional dominance' from the haploid parent was observed.

- Despite major genomic and nucleo-cytoplasmic disruptions, enhanced fitness was detected in the triploid strain. By comparing gene expression across generations, our results indicate that proteostasis restoration is a critical component of rapid adaptation following genome merging in *Chlamydomonas reinhardtii* and possibly other systems.



Challenging reproductive barriers between and within species: the role of ploidy in the genesis of new species

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POSTER SESSION 1 - P01.146, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Whole genome duplication (polyploidy) is a widespread phenomenon in nature and a key driver of plant evolution and diversification. However, newly formed polyploids (neopolyploids) often struggle to establish themselves due to frequency-dependent selection, where the more common parental cytotype inhibits their spread, a concept known as the minority cytotype exclusion hypothesis. Polyploidy typically leads to immediate reproductive isolation, but the role of triploids, which arise in diploid-tetraploid complexes, remains ambiguous. Theoretical models propose two contrasting roles for triploids: the "triploid block," which inhibits the establishment of new polyploids due to low fertility, and the "triploid bridge," which facilitates the formation of viable polyploid populations by producing viable gametes. Despite their theoretical importance, the frequency and conditions under which triploids act as blocks or bridges remain poorly understood. This study aims to evaluate the strength of reproductive barriers within and between different ploidy levels and species, using hand-pollination experiments in a common garden setting.

Different cytotypes of *Jasione maritima* and *Jasione montana*, two Mediterranean species from the Campanulaceae family with naturally occurring diploid and tetraploid individuals, were used as a model system. The pollination treatment consists of inter-cytotype crosses within and between species and intra-cytotype crosses within and between species with each cytotype acting as both pollen donor and recipient. Crosses were performed using three populations of each species and cytotype. To assess the reproductive success and isolation among cytotypes we examined fruit and seed production, seed germination rates and offspring ploidy. We hypothesize that whole genome duplication generates reproductive isolation comparable to that observed between species i.e., ploidy generates new entities reproductively isolated from parentals, the first step towards speciation. Our findings provide insights into the early stages of reproductive isolation in polyploid complexes, advancing our understanding of how polyploidy drives plant diversification and speciation.



Chromosomes, the more the merrier?

How does tetraploid *C. elegans* resist severe cold stress at the gravid adult stage?

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POSTER SESSION 1 - **P01.150**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Whole genome duplication (WGD), or polyploidy, involves the duplication of an organism's entire genome and plays a major role in evolution. In early vertebrates, two rounds of WGD have coincided with mass extinction events, suggesting potential advantages in stressful environments. In plants, WGD is a well-established mechanism of speciation and stress resistance. In contrast, its role in animals remains unclear due to the rarity of WGD events.

Our research investigates the consequences of polyploidy and its potential short-term adaptive advantages under stress — a topic rarely explored in animals. We use *Caenorhabditis elegans*, where tetraploidy can be induced experimentally. Although tetraploid *C. elegans* display reduced fitness, fertility, and lifespan under normal conditions, they show improved survival and increased reproductive output following severe cold shock, suggesting a possible advantage under stress.

This project focuses on the role of lipid dynamics and reproductive status during and after cold shock. In diploid gravid adults, prior data in diploids suggest that vitellogenin-dependent lipid relocation from the intestine to embryos may contribute to post-severe-cold-stress mortality. Furthermore, we find that the survival of gonad-less diploid worms upon recovery from cold stress (CS) is similar to that of their tetraploid counterparts. We hypothesise that tetraploid worms survive upon recovery from CS, either because of metabolism/lipid localisation alterations, and/or germline alterations. We investigate how the lipid dynamics occurring in tetraploids compare to the ones in diploids, and whether cold-

induced mortality is influenced by specific reproductive factors such as germline activity and reproductive state.

To examine lipid distribution during recovery, we perform BODIPY lipid staining on cold-shocked wild-type diploid and tetraploid *C. elegans*. This allows us to visualize lipid localization patterns and assess whether tetraploids exhibit differences in intestinal lipid retention or redistribution compared to diploids, which could underlie their increased survival and reproductive output.

To further assess the contribution of reproductive factors to cold-induced death, e.g. whether the absence of embryos or fertilization alters sensitivity to cold, we use three *C. elegans* mutants: *fem-3(q20)*, which produces oocytes but is deficient in spermatogenesis, *fog-2(q71)* which comprises feminised XX individuals and WT XO males and *glp-1(e2141)*, which lacks germ cell proliferation.

Certain reproductive processes (allocation of lipids to the embryos, gonads integrity or germline proliferation) may contribute to cold-induced mortality, and their disruption could reduce the cost of reproduction under stress. If confirmed, this would support the notion that tetraploid resistance involves altered reproductive investment or changes in lipid trafficking. The outcomes of this study may reveal new mechanisms by which polyploidy buffers environmental stress and offer insights into the reproductive costs of extreme conditions.

Overall, this work seeks to connect cold stress survival with reproductive physiology and lipid metabolism, offering new perspectives on the adaptive potential of polyploidy in animals.



Consequences of induced whole genome duplication on the memory of environmental stress in tetraploid *C. elegans*

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POSTER SESSION 1 - **P01.157**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Polyploidy is the result of a dramatic mutational event, the duplication of the entire genome. Whole genome duplication (WGD) is a natural phenomenon, particularly common and recognised as a major evolutionary force in plants. In animals, recent polyploid species are rarer, and the evolutionary advantage of WGD is more debated, as polyploidy also comes with substantial challenges.

Increasing evidence suggests that WGD-induced changes in cell and nuclear geometry are critical factors contributing both to challenges and to potentially beneficial novelties observed in neopolyploids. Decreased nuclear surface-to-volume ratio, for example, can influence chromatin organization, and consequently, the distribution of repressive histone marks. Moreover, nuclear geometry is also directly linked to the regulation of stress responsive genes.

This research aims to investigate the short-term consequences of WGD on the epigenetic landscape of tetraploid animals and the outcomes for the memory of stress responses. Using *Caenorhabditis elegans* as a model, we are able to experimentally induce WGD and obtain genetically identical tetraploid animals from almost any diploid strain. So far, studying autotetraploid *C. elegans* revealed that, although tetraploidy reduces fitness in regular conditions, tetraploids show increased resistance under temperature-related stress conditions, but is

the memory of such environmental stress affected after WGD?

Individuals exposed to a small dose of stress mount a stronger response when re-exposed to a higher dose of stress, a phenomenon called hormesis. While heat hormesis seems to be unaffected by WGD, we found the expression of a heat sensitive multicopy transgene to be increased in tetraploids. Measuring the fluorescence levels of this multicopy transgene allows to (indirectly) observe the behaviour of heterochromatin, specifically that of repeated elements. Moreover, temperature-induced changes in the expression of this array have been shown to be heritable across several generations. Therefore, observing increased expression levels of this multicopy array following WGD led to the hypothesis that its inheritance might also be affected. Our preliminary experimental data suggest that WGD might in fact alter the transgenerational memory of heat stress, through de-repression of repeated elements. Coupled with this, immunofluorescence on key histone modifications, along with qRT-PCR of endogenous repeated elements (e.g., transposons), will provide further information on whether the distribution of repressive histone marks is altered in tetraploid worms.

This work aims to link WGD-induced nuclear perturbations and altered stress responses in polyploid animals, potentially through epigenetic remodelling and changes in the distribution of histone modifications.



Dynamic Transposon Activation and Gene Regulatory Rewiring Drive Functional Divergence of Duplicated Genes During the Rediploidization Process in *Orychophragmus violaceus*

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POSTER SESSION 1 - **P01.144**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: The regulatory and functional diversification and innovation that follow whole-genome duplication (WGD) are essential processes driving long-term evolution, particularly during the extended rediploidization process. Additionally, the dynamic activity of transposable elements (TEs) often coincides with WGD and rediploidization, playing diverse roles in genome evolution. TEs act not only as sources of genomic instability but also as drivers of regulatory innovation. However, our understanding of the evolutionary dynamics of duplicated genes and the mechanisms through which TEs contribute to gene regulatory innovation during rediploidization remains limited. *Orychophragmus violaceus* underwent a lineage-specific WGD approximately 13.4 million years ago and now possesses one of the largest genomes in the Brassicaceae family, largely due to a high proportion of transposon-derived sequences. This makes *O. violaceus* an excellent model for exploring the interplay between TE activity, gene regulatory evolution, and functional divergence during rediploidization.

Method and Materials: In this study, we de novo assembled a chromosome-level reference genome for *O. violaceus* and generated complementary multi-omics datasets from multiple tissues, including RNA-seq, whole-genome bisulfite sequencing (WGBS), and ATAC-seq from leaves, stems, roots, flowers, and siliques. Additionally, we obtained high-depth whole-genome resequencing data from 12 wild individuals for population genomic analyses.

Using these datasets, we quantified expression, DNA methylation, and chromatin accessibility divergence between duplicated genes following WGD, comparing these patterns with those observed in outgroup species. Furthermore, we identified genes exhibiting tissue-specific sub- and neo-functionalization and deciphered the mechanisms underlying their potential functional divergence. This was achieved by integrating multiple experimental validations, including luciferase reporter (LUC) assays, electrophoretic

mobility-shift assays (EMSA), and β -glucuronidase (GUS) assays.

Results: First, we observed a general bias toward reduced expression levels in paralogous genes following whole-genome duplication (WGD), likely as a mechanism to maintain gene dosage balance after ploidy changes. The downregulated genes were primarily associated with a high abundance of nearby transposable elements (TEs) and increased DNA methylation within gene bodies across CG, CHG, and CHH contexts.

Second, the analysis of duplicated genes revealed widespread divergence in expression levels, DNA methylation patterns, and chromatin accessibility between paralogs. Relaxed selective constraints were associated with both the rapid expression and sequence divergence of these gene pairs. Notably, compared to its paralog, which retained a conserved ancestral expression pattern, one of the duplicated diacylglycerol acyltransferase (DGAT) genes exhibited significantly higher expression in seeds, likely linked to the newly identified 24-carbon dihydroxy fatty acids found specifically in *O. violaceus*.

Lastly, we found that active TEs are strongly correlated with the divergence or emergence of new cis-regulatory elements, which are further linked to tissue-specific sub- and neo-functionalization of duplicated genes. Notably, using LUC, EMSA, and GUS assays, we experimentally validated a TE-associated, newly emerged flower-specific accessible chromatin region upstream of a copy of the Cinnamoyl-CoA reductase 2 gene (Ov-CCR2). This region contained a novel binding motif that can be preferentially activated by Ov-WRKY70, providing clear evidence that TE-associated regulatory innovation drives the functional divergence of duplicated genes.

Conclusion: Our findings demonstrated and experimentally validated that dynamic transposon activation during the rediploidization process played a pivotal role in rewiring gene regulatory evolution and driving functional innovation following whole-genome duplication in *Orychophragmus violaceus*.



Evidence for lineage-specific rediploidisation following the 1R whole-genome duplication at the base of the vertebrate lineage

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POSTER SESSION 1 - **P01.159**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Whole genome duplications (WGDs) - dramatic events in which the entire genome of an organism is doubled - have occurred multiple times in the evolutionary history of plants and animals. Two rounds of WGD (termed 1R and 2R) occurred at the base of the vertebrate lineage, with 1R occurring before the divergence of jawless and jawed vertebrates (cyclostomes and gnathostomes), and 2R being gnathostome-specific. A hexaploidisation event also happened in cyclostomes. WGDs provide much raw material for evolution and may have facilitated lineage survival during mass extinctions.

Following WGD, alleles are inherited tetrasomically. Only after the genome has returned to diploid, through the process of rediploidisation, can gene duplicates (ohnologs) diverge in sequence and function. Rediploidisation has been shown in some lineages to be a delayed and asynchronous process, occurring over millions of years, with cases where species divergence has occurred before rediploidisation is complete (Redmond et al., 2023).

We investigate evidence for lineage-specific rediploidisation following 1R, which previous studies have not supported. Lineage-specific rediploidisation allows for independent divergence of ohnologs resulting from the same WGD, and evolution of lineage-specific novelties, which may have interesting implications for the evolution of gnathostome genomes, including humans.

Materials and methods: We improve upon previous analyses, using recently released hagfish genomes, and utilising a high-confidence ohnolog dataset. We generate proto-vertebrate, proto-gnathostome, and proto-cyclostome reconstructions, incorporating genomic data from additional species compared to previous analyses. We combine topology and this macro-synteny

information for tree classification, and test for biases between pre- and post-speciation tree sets including in compositional heterogeneity.

Results: We successfully use macro-synteny information combined with tree topologies to classify previously uninformative trees featuring extensive gene loss, substantially increasing the amount of tree data available for investigation. We find evidence for lineage-specific rediploidisation following 1R, recovering high-confidence sets of pre-speciation and lineage-specific rediploidised ohnologs. Clear clusters by ohnolog type (pre-speciation or lineage-specific) are seen when these genes are mapped onto hagfish chromosomes, and chromosomes of gnathostomes, including humans. This adds confidence to our classifications and provides further evidence for lineage-specific rediploidisation following 1R.

Conclusions: We find evidence for lineage-specific rediploidisation following 1R. Our findings indicate regions of the genome had not rediploidised prior to the gnathostome-cyclostome split. As this allows for independent divergence of such ohnologs, lineage-specific rediploidisation may have facilitated the evolution of lineage-specific novelties seen in cyclostome and gnathostome genomes. Enrichment analysis and investigation and comparison of functions of pre- and post-speciation rediploidised genes may shed further light on the potential impact of delayed rediploidisation on vertebrate evolution.

References: Redmond, A.K., Casey, D., Gundappa, M.K. et al. Independent rediploidization masks shared whole genome duplication in the sturgeon-paddlefish ancestor. *Nat Commun* 14, 2879 (2023). <https://doi.org/10.1038/s41467-023-38714-z>



Genomic and functional mechanisms underlying early adaptation to whole genome duplication per se.

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POSTER SESSION 1 - P01.156, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Whole-genome duplication (WGD) – resulting in polyploidy – has repeatedly occurred over evolutionary time, affecting all living organisms and particularly plants. WGD has been associated with major biotic and abiotic crises. Environmental stressors such as extreme temperatures tend to induce WGD. In turn, polyploid organisms typically show higher and/or broader tolerance to these stresses, enabling them to efficiently colonize suitable niches, even extreme habitats. However, WGD poses a serious challenge in the early stage of polyploid evolution, as it disrupts fundamental cellular processes and especially meiotic pairing and segregation. In nascent autopolyploids (within-species WGD), the presence of multiple homologous chromosomes may result in multivalent formations leading to instable meiosis and resulting in severe chromosome rearrangements or damage. Interestingly, meiotic instability seems often resolved in established autopolyploid populations while meiosis remains highly instable in neo-autopolyploids. Unfortunately, this has been poorly investigated in natural neo-

autopolyploid populations due to the scarcity of suitable model species. *Mimulus guttatus* (the “monkey flower”) is an emerging model species that has been studied for over 50 years due to its wide diversity of floral traits but also for its wide array of ecological and life traits. *M. guttatus* is native from America, but it has been introduced in Europe in the 19th century. Recent studies have found several autopolyploid populations along with known diploid ones in both the native and the invasive range. This sets up the perfect ground to investigate the genomic and functional mechanisms underlying the early adaptation to WGD, as well as to validate the role of meiotic stability in the evolutionary success of polyploidy in nature. To do that, we compared diploid and autotetraploid populations of *M. guttatus* between the native and the invasive range of the species, based on resequenced short-reads data and whole-genome scans. We identified several candidate genes and further characterized the impact of their genomic mutations to their resulting protein structure and function.



How does the common barbel (*Barbus barbus*) see? The effect of the whole-genome duplication on vision

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POSTER SESSION 1 - P01.143, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Teleost fishes outstand among all vertebrates in the number of visual pigments and photoreceptor types. This extraordinary development was also facilitated by the teleost-specific whole genome duplication that occurred approximately 350 Mya. Here we explore teleost species that recently underwent subsequent whole genome duplication – the common barbel (*Barbus barbus*). This event was accompanied by hybridization (i.e., it was allopolyploidization) and resulted in 14 opsin genes found in the genome, an unusually high number even among teleosts. Does the expansion of opsin gene repertoire result in better visual abilities? We show opsin expression profiles of adult specimens and larvae at different developmental stages. Within three opsin classes (UV-sensitive SWS1, blue-sensitive SWS2, and green-sensitive RH2), we found opsin pairs with a clear temporal division of function – larval

and adult genes. Furthermore, the switch in expression from larval to adult gene copy in these opsin classes was synchronized – such effect has never been reported for a polyploid species. We investigated if such a division of function can be adaptive. We predict which opsin proteins differ in spectral sensitivity based on the amino acid substitutions in the key tuning sites. Are absorption spectra of opsins tuned to match the light available in the environment? We localise and visualise the opsin gene expression in adult retinae via fluorescence in situ hybridization and discuss its functional consequences – namely if the presence of more opsins in the retina enhances colour vision or sensitivity of the visual apparatus. Finally, we present the latest results of single-nuclei RNA sequencing performed on the adult retina to reveal how individual cells utilise vision-related genes acquired by the polyploidization event.



Metabolic and physiological responses to genome duplication in duckweed: Linking polyploidy, size, and stress

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POSTER SESSION 1 - **P01.152**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Whole genome duplication (WGD), or polyploidy, is a key evolutionary process that frequently alters plant physiology, morphology, and ecological responses. Though polyploid individuals arise commonly in nature, the successful establishment of polyploid populations remains rare. This paradox—frequent formation but infrequent persistence—raises important questions about the physiological and ecological consequences of genome duplication, particularly under environmental stress.

One consistent effect of WGD is increased cell and body size, which may modify a plant's metabolic rate and growth strategy. These changes could either confer tolerance to stress or impose unsustainable energetic demands, depending on context. Understanding how genome duplication affects metabolic traits and stress responses is essential for evaluating the ecological viability of polyploids and their potential to coexist or outcompete diploid progenitors.

In this study, we focus on *Spirodela polyrhiza*, an aquatic duckweed with rapid clonal growth and a well-characterized diploid background. We experimentally investigate how genome duplication influences size, biomass accumulation, and CO₂ exchange under salt stress, using a common garden approach across multiple genetic backgrounds. Our work builds on a prior study of neotetraploids derived via colchicine treatment, expanding the experimental design to nine diploid genotypes and their respective polyploids from three separate polyploidization events.

Each diploid–tetraploid pair was grown under a gradient of salt concentrations to simulate increasing osmotic stress. We measured fresh and dry biomass and recorded gas exchange data using the TARGAS-1 portable photosynthesis system and CO₂ consumption as a proxy for metabolic activity.

This expanded dataset allows us to assess not only the overall effects of genome duplication, but also how genetic background modulates those effects. Specifically, we ask whether neopolyploids consistently exhibit increased size, altered metabolic rates, or enhanced tolerance to salt, and whether these traits depend on genotype or stress level. We also evaluate whether gas exchange patterns scale with individual size or biomass-normalized activity.

Preliminary findings suggest that genome duplication leads to larger frond size and higher individual CO₂ assimilation, but not necessarily to improved salt tolerance. However, strain-specific responses and scaling patterns may reveal more complex genotype-by-ploidy interactions. These results will be presented and discussed in the context of eco-evolutionary dynamics and polyploid establishment theory.

By combining detailed physiological data across genotypes and stress conditions, this study provides a robust experimental test of theoretical predictions about polyploid performance. It also lays the foundation for future work linking size–metabolism relationships to the ecological success of polyploids in fluctuating environments.



Ploidy-dependent differences in mutation rate, spectrum and adaptive outcomes in *Chlamydomonas reinhardtii*

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POSTER SESSION 1 - P01.139, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Mutation is the primary source of genetic variation and as such a prerequisite for evolutionary change. One of the most spectacular mutations, Whole genome duplication (WGD), has a high initial cost but is nevertheless ubiquitous within plants and is associated with evolutionary diversification and speciation. Classic population genetic models at the basis of our understanding of ploidy related differences in adaptation rate, often assume a duplication of the mutation rate, but studies on yeasts show that this assumption is overly simplistic and that, for instance, genetic background and heterozygosity can affect mutation rate. Despite the evolutionary importance of WGD in plants, the impact of WGD on the mutation rate and spectrum in the 'green lineage' is understudied. To address this gap, we conducted a mutation accumulation (MA) experiment with the

unicellular plant *Chlamydomonas reinhardtii*. Our study included 20 mutation accumulation lines from four haploid genotypes and 20 diploid mutation accumulation lines from four different genotypes with varying levels of heterozygosity. Over 14 months covering about 1000 mitotic generations, all 160 MA lines were grown under relaxed selection allowing the accumulation of all non-lethal mutations. The end points were sequenced and mutations were called. The results were integrated with fluorometry-based genome size measurements and data on the fitness evolution, and allow us to determine ploidy-related differences in mutation rate and spectrum including genomic (in)stability, aneuploidies, and the impact of heterozygosity, and ultimately, how these affect fitness.



Polyploidy in Action: Disentangling the Consequences of Genome Doubling in *Arabidopsis arenosa*

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POSTER SESSION 1 - P01.145, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Whole-genome duplication (WGD) is a key evolutionary process in flowering plants, often linked to changes in phenotype, life history, and stress responses. Yet, distinguishing immediate effects of genome doubling from traits shaped by long-term evolution remains challenging. *Arabidopsis arenosa*, which occurs naturally as diploid and tetraploid lineage, offers a valuable model. By comparing natural diploids, established tetraploids, and lab-induced neo-tetraploids, we aimed to separate the direct consequences of WGD from evolved features of stabilized polyploid lineages.

Using a common garden approach, we phenotyped a range of macroscopic, life-history, fitness, and cellular traits in the ancestral diploids and both tetraploid cytotypes. Neo-tetraploids produced bigger leaves but fewer flowers and marginally fewer but heavier seeds, indicating early trade-offs of WGD. Among cellular traits, endoreduplication (DNA replication without cell division) exhibited significant variation.

Its levels were similar in diploids and neo-tetraploids but significantly reduced in natural tetraploids, suggesting that the decrease in endoreduplication is not a direct effect of WGD but likely evolves over time under post-WGD selection.

To address whether WGD provides greater plasticity and evolvability, we exposed plants to drought stress in a short-term directional selection experiment. Rosette diameter and leaf area were significantly reduced across all cytotypes, while endoreduplication remained stable, reinforcing its role as a robust, non-plastic trait. To directly assess evolvability of the experimental populations, a second generation of plants is currently being grown under the same conditions, with the aim of quantifying trait shifts between generations using the breeder's equation. This approach will allow us to test whether WGD per se alters the evolutionary potential of plant populations in response to environmental stress.



Population genomic evidence of inter-ploidy gene flow in natural plant populations

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POSTER SESSION 1 - **P01.155**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Polyploidy, the presence of multiple genome copies as a result of whole-genome duplications, is often thought to cause immediate reproductive isolation between diploids and related polyploids due to sterility and inviability of inter-ploidy offspring. However, cytological and population genetic evidence suggests that gene flow between plants with different ploidy levels (introgression) can happen in natural populations. Such inter-ploidy gene flow can happen either via production of viable gametes of triploid hybrids (triploid bridge) or, more commonly, via one-step gene flow mediated by unreduced gametes of a diploid. Recent research demonstrated first genomic and population genetic evidence consistent with hybridization and backcrossing in natural populations of diploid and tetraploid populations of multiple plant systems, challenging the paradigm of an impermeable ploidy barrier. Yet, it remains unclear how common such inter-ploidy introgression is and to what

degree it contributes to adaptation. Here we present preliminary evidence from multiple naturally occurring angiosperm lineages, where diploids and related autotetraploid lineages form one or multiple secondary contact zones. We use whole-genome resequencing data to detect evidence of inter-ploidy gene flow by using multiple population genomic analyses, which compare excess sharing of variation between diploids and tetraploids from the contact zones with populations from regions in which either only diploids or only tetraploids are located. Using this approach we test for genome-wide patterns of inter-ploidy gene flow. In addition, we identify local genomic patterns, with the aim to identify genomic regions where natural selection may favor or suppress inter-ploidy gene flow and ultimately test if such patterns are shared across lineages, in which polyploidy and secondary contact with diploids evolved independently.



Sex, behavior, and giant cells: missing elements of animal polyploid evolution with the parasitoid *Nasonia vitripennis*

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POSTER SESSION 1 - **P01.149**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Ancestral whole genome duplication occurred in most eukaryotic lineages, with consequences at levels spanning individual physiology to adaptive advantage of species and populations. However, there are many gaps in the empirical study on animal polyploid evolution. This is because polyploidization, while challenging for all taxa, is especially detrimental for animals. They have severe immediate problems of cellular gigantism; sterility and gametic incompatibility; and disruptions to gene expression including sex dosage effects. These have limited research to single generations, or asexual lineages that can self-reproduce. We recently developed the sexual parasitoid wasp *Nasonia vitripennis* as a comprehensive model to overcome these barriers in animal polyploid study. An easy-to-maintain system, polyploids can reliably be induced with meiotic inhibition or gene silencing of various targets in the sex determination pathway. Importantly and exceptionally, *Nasonia* polyploids of both sexes are reproductive, enabling continuous study through generations. Extensive foreknowledge of the *Nasonia* system also allows for more complex contextualization of animal polyploidy. Our studies are the first indication of sexual tradeoffs in polyploid evolution, as lines that exhibit high male

reproductive success (mate acceptance and offspring production) correspondingly have low female reproductive success (offspring production), and vice versa. Complicating this is the influence of behavior, with strong female mate choice possibly discriminating against impaired polyploid males, so inhibiting inheritance of the polyploid state. One suspect factor is somatic endopolyploidy: regional giant, multinucleated cells obligate to normal development. How aberrant organismal polyploidization (e.g., its own cell gigantism effects) impacts endopolyploidy is largely unknown, but *Nasonia* males have endopolyploid wings key to courtship success. The retention of endopolyploidy is inconsistent in polyploid males, likely correlating to their varying mating success. And yet, *Nasonia* males also have sneaking behavior, allowing disadvantaged polyploid males to benefit from non-polyploid male signals and enhance their mating success. This highlights the need to examine behavioral interactions for their contribution the evolutionary successes and failures of polyploid lineages. This collective research in the *Nasonia* model reflects a new phase of animal polyploid evolution study accounting for interrelated roles of sex, behavior, and endopolyploidy.



Single-cell sequencing suggests a conserved function of Hedgehog-signaling in spider eye development

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POSTER SESSION 1 - P01.138, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Spiders evolved different types of eyes, a pair of primary eyes that are usually forward pointing, and three pairs of secondary eyes that are typically situated more posterior and lateral on the spider's head. The best understanding of arthropod eye development comes from the vinegar fly *Drosophila melanogaster*, the main arthropod model organism, that also evolved different types of eyes, the larval eyes and the ocelli and compound eyes of the imago. The gene regulatory networks that underlie eye development in this species are well investigated revealing a conserved core network, but also show several differences between the different types of eyes. Recent candidate gene approaches identified a number of conserved genes in arthropod eye development, but also revealed crucial differences including the apparent lack of some key factors in some groups of arthropods, including spiders.

Materials and Methods: Parasteatoda tepidariorum embryos at mid-late developmental stages were processed for single-cell RNA sequencing following the 10x Genomics Single Cell 3' protocol at the Department of Developmental Biology and Gene Core facilities of the European Molecular Biology Laboratory, Heidelberg, Germany. Sequencing reads were mapped to the reference genome using 10x Cell Ranger pipelines and the resulting gene expression matrix was analysed with the bioinformatic tool 'Seurat'. Cell clustering was validated via in situ hybridisation and Gene Ontology analysis. Embryos of related spider species (namely, *Acanthoscurria geniculata*, *Ischnothele caudata*, *Pholcus phalangioides*, and *Pardosa amentata*) and the harvestman *Phalangium opilio* were fixed and used for in situ hybridisation to visualize the expression patterns

of hedgehog orthologs and selected gene markers that were found in the scRNA data. Sequence alignment and Phylogenetic analysis were performed using T-coffee and Mr. Bayes, respectively.

Results: Here, we re-analysed our published scRNA sequencing data (Medina-Jiménez et al. (2024) Single-cell RNA sequencing of mid-to-late stage spider embryos: new insights into spider development) and found potential key regulators of spider eye development that were previously overlooked. Unlike earlier research on this topic, our new data suggest that Hedgehog (Hh)-signalling is involved in eye development in the spider *Parasteatoda tepidariorum* via a second paralog (hh2). By investigating embryonic gene expression in representatives of all main groups of spiders, we demonstrate that this involvement is conserved in spiders. The most likely scenario in spiders is that the two copies of hedgehog have undergone sub-functionalization after a duplication that occurred in the lineage leading to Arachnopolmonata (i.e. scorpions, whip spiders, and spiders), and indeed similar cases of sub-functionalization in spiders have been recorded frequently (e.g. Schwager et al., 2017; Leite et al, 2018; Janssen et al., 2021; Aase-Remedios et al., 2023).

Additionally, we identified genes that are expressed in the developing eyes of spiders, but that have not been studied in this context before. **Conclusion:** Our data show that single-cell sequencing represents a powerful method to gain deeper insight into gene regulatory networks that underlie the development of lineage-specific organs such as the derived set of eyes in spiders. Overall, we gained deeper insight into spider eye development, as well as the evolution of arthropod visual system formation.



The phased genome assembly of *Xenopus mellotropicalis* sheds light on the pace of subgenome dominance and diploidization in animal polyploids

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POSTER SESSION 1 - **P01.148**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Polyploidization plays a significant role in driving macroevolution. Yet, its evolutionary consequences remain poorly understood in animals, where polyploidy is rare, understudied and often rapidly followed by rediploidization. In frogs, *Xenopus* is one of the few genera with widespread polyploidization, with species ranging from diploid ($2n = 2x$) to dodecaploid ($2n = 12x$). We present a high-quality, phased genome assembly of the tetraploid frog *Xenopus mellotropicalis*, constructed from Nanopore, Illumina, and Hi-C sequencing reads. The 2.98 Gbp genome comprises 20 chromosomes, with a BUSCO completeness score of 97.2% (88.9% duplication), a scaffold N50 of 157 Mbp, and a contig N50 of 914 Kbp. Using k-mer analysis, we successfully phased the two subgenomes. Gene and transposable element (TE) annotations revealed that subgenome A harbours more genes and fewer TEs than subgenome B. Expression profiling showed higher expression in subgenome A,

consistent with ongoing subgenome dominance. Kimura divergence of ohnologue pairs suggests a recent polyploidization event (~ 9 million years ago), classifying *X. mellotropicalis* as a neopolyploid. Gene and genome comparison between the *X. mellotropicalis* and the diploid *Xenopus tropicalis* revealed that both subgenomes are highly syntenic with *X. tropicalis*. Interestingly, genes from subgenome B are phylogenetically closer to *X. tropicalis*, though about one-third of genes show the opposite pattern. This indicates that homoeologous exchange might occur during its diploidization, contrasting with previous observations in the allotetraploid *Xenopus laevis*, where homoeologous exchanges are scarce. Our results provide new insights into diploidization and subgenome evolution in animal polyploids, highlighting *X. mellotropicalis* as a valuable model for studying genome evolution after a relatively recent allopolyploidization.



Transposable Elements as Drivers of Regulatory and Genomic Innovation in Spiders

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POSTER SESSION 1 - P01.153, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Gene and genome duplications provide new raw material for novel gene functions and regulatory networks and are thus key mechanisms of evolutionary diversification. Recent comparative genomics analyses suggest a large-scale or even whole-genome duplication event in the common ancestor of spiders and scorpions, which could have set the stage for spider-specific innovations, such as silk and venom production, as well as specialized respiratory systems. Despite growing interest, the molecular mechanisms shaping expression and regulatory divergence after duplication remain poorly understood in arthropods.

Transposable elements (TEs)—mobile repetitive sequences known to influence genome architecture and regulation—have been shown to play a role in gene expression divergence following duplication events. To link TEs to gene duplication diversification in spiders, we established curated TE libraries for 24 chelicerate species, including spiders, scorpions, and ticks. We show that constructing species-specific TE libraries are essential to obtain reliable TE annotations and to infer TE activities through evolutionary time. Using our curated libraries, we investigated the relationship between repeat content and gene architecture, revealing a strong positive correlation between intron length and TE occupancy—suggesting that transposable element (TE) accumulation contributes to intron expansion. Since

introns often contain regulatory information, we hypothesized that intron expansion could provide a substrate for regulatory divergence between gene duplicates. We are testing this hypothesis by integrating the occurrence of intronic TEs with functional genomics data for the two distantly related spider species *Parasteatoda tepidariorum* and *Ischnothele caudata* to assess gene regulation (ATAC-seq) and gene expression (RNA-seq) between duplicated genes. In addition to their presence in non-coding regions, TEs can also be exonized to contribute to divergence on the protein level. A preliminary analysis identified more than 3400 TE-derived distinct exonic sequences in a spider, pointing to a potentially significant contribution of TEs to coding sequence evolution. While exonized TEs have been documented in vertebrates, there is a notable lack of systematic investigation into their role in arthropods, making these findings a promising starting point for future exploration in this group.

In summary, our data demonstrates the value of tailored TE annotation strategies for non-model species, enabling high confidence downstream analyses of TE distribution, function, and evolutionary relevance. We employ our spider-specific TE annotations to link TE activity to diversification of gene regulation and protein evolution after gene duplication.



Zombie Gene Flow: Asexual Polyploid Hybrids Reintroduce Genomic Elements of Extinct Species into Contemporary Sexual Populations

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POSTER SESSION 1 - **P01.158**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Whole genome duplications (WGD) and hybridization are widely recognized as potent drivers of evolutionary novelty, often linked to bursts of species diversification, genomic innovation, and adaptation. However, prevailing paradigms largely focus on sexually reproducing taxa, assuming that interspecific gene flow requires fertile, recombining hybrids to transmit alleles between gene pools. Hybrids with non-standard reproductive modes, such as clonal or odd-ploidy forms, are typically viewed as evolutionary dead ends.

Here, we challenge this view by presenting the first fully documented case of gene flow from an extinct species into an extant sexual lineage, mediated not by meiotic recombination, but by asexual, polyploid hybrids. Using cytogenetic, experimental, and phylogenomic data, we show how asexual diploid-triploid hybrids in *Cobitis loaches* act as “zombie gene flow” vectors, reintroducing long-lost genomic elements from extinct ancestors into contemporary sexual populations.

Specifically, we uncover how ancient hybridization between the now-extinct sexual species (“X”) and the extant *Cobitis ohridana* (“O”) gave rise to diploid (OX) and triploid (OOX) hybrids, which differ sharply in reproductive strategy. Diploid OX hybrids reproduce mostly clonally, preserving the X genome intact over evolutionary time. However, occasional gene conversion events ingress X-alleles onto O-genomic background. By contrast, triploid OOX hybrids systematically eliminate the X genome during gametogenesis, but generate reduced gametes by recombining O chromosomes, allowing them to backcross with sexual *C. ohridana*. This ploidy-dependent oscillation between clonal maintenance and sexual recombination enables substantial gene flow from the extinct X lineage into the

contemporary O gene pool, resulting in over 4% genomic admixture detectable in nuclear and mitochondrial compartments of modern *C. ohridana*.

Importantly, while our system offers the first full empirical demonstration of “zombie gene flow,” its mechanistic components—clonal maintenance, ploidy variation, occasional rediploidization, and cryptic recombination—are shared across diverse taxa, including fish, amphibians, insects, and plants. This suggests that polyploid hybrid systems worldwide may harbor a hidden capacity to preserve and reintroduce ancient genetic material, reshaping our understanding of WGD’s role in macroevolutionary dynamics.

Our work also touches on key themes in the study of genome evolution: the long-term retention of duplicated genomic material, shifts in gene expression regulation following hybridization and WGD, and the evolutionary consequences of genome rearrangement and rediploidization. Notably, we highlight how hybrid asexuals can act as cryptic reservoirs of genomic variation, challenging assumptions about the permanence of extinction and offering new insights into how WGD and polyploidy can shape the genetic architecture of biodiversity.

Finally, we emphasize the conservation implications of our findings. As global species loss accelerates, recognizing the evolutionary potential of hybrid asexuals to preserve genetic legacies from extinct taxa opens new avenues for biodiversity conservation and management. By integrating cytogenetics, experimental crosses, and comparative genomics, our study not only redefines the evolutionary roles of polyploidy and asexuality but also calls for a broader, integrative framework to capture the hidden contributions of WGDs to long-term evolutionary resilience.



Breaking the ploidy barrier: Genomic insights into allopolyploidy and cross-ploidy hybridisation in British plant genus *Euphrasia* (eyebrights).

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MEETING ROOM 118+119, August 18, 2025, 2:00 PM - 5:00 PM

Polyploidy, or whole genome duplication (WGD), has long been recognised as an important driver of speciation, as it is widely assumed to cause instantaneous reproductive isolation between newly formed polyploids and their diploid progenitors. However, recent research has increasingly documented cross-ploidy hybridisation across a range of taxa, highlighting the potential of interploidy hybridisation to shape evolutionary outcomes. In this study, I investigate the role of cross-ploidy hybridisation in British native *Euphrasia* (eyebrights), a genus of diploids and tetraploids that frequently hybridise. Using one of the first high quality polyploid reference genome from the Darwin Tree of Life project—which is generating reference genomes for all eukaryotic species in Britain and Ireland—I show polyploid *Euphrasia* are

allopolyploid in origin, with divergent subgenomes. Using genome resequencing data from 118 diploid and tetraploid *Euphrasia* samples, I show subgenome A is genetically divergent from extant diploid *Euphrasia* species while subgenome B shows genetic similarity to existing diploids. Preliminary sequence comparisons of these diploid and tetraploid populations reveal clear groupings according to ploidy, but notably there are some intermediate individuals consistent with rare cases of interploidy gene flow. These results underscore the evolutionary potential of interploidy hybridisation, with future work to assess whether adaptive introgression occurs repeatedly across a ploidy barrier and underlies the origin of the cross ploidy hybrid species *Euphrasia vigursii*.



Consequences of whole-genome duplication on expansion and adaptation to environmental heterogeneity in *Biscutella laevigata*

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MEETING ROOM 118+119, August 18, 2025, 2:00 PM - 5:00 PM

Whole-genome duplication (WGD) is a major driver of plant speciation and often considered to promote rapid adaptation to new or changing environmental conditions. However, the extent to which WGD per se fosters phenotypic novelties, how diploids vs polyploids respond to selection, and how long-term evolution act on WGD-induced changes remain unclear. To investigate those long-standing questions, the *Biscutella laevigata* textbook example of mixed-ploidy system is a great model. Climatic niche modelling together with spatial genetics and coalescent modelling highlighted that diploids restricted to never-glaciated lowlands were pushed out of the Alps during the Pleistocene and differentiated for the last 100 KY, whereas tetraploids occurring at high-elevation in previously-glaciated areas were formed before the last glacial maximum (ca. 25 KY ago) from a single diploid population and then recolonized the Alps. Tetraploids display tetrasomic inheritance and occupy similar environmental niche as their diploid progenitors, indicating autopolyploidy (or WGD s.str).

To characterize the relative contribution of WGD per se vs post-WGD evolution to phenotypic and transcriptomic changes, our work compared diploid, synthetic tetraploid and established tetraploid lines of *B. laevigata*, assessing phenotypic plasticity and stress resilience following growth under either stable or changing conditions (i.e. involving a daily temperature stress). While WGD directly induced some phenotypic changes, transcriptional changes were driven by subsequent evolution rather than by WGD itself. Post-WGD evolution indeed modulated WGD-induced phenotypic changes by either reverting them toward diploid-like values or

maintaining them. Finally, WGD led to a reduction of survival and fitness, which was mitigated by post-WGD evolution, resulting in established tetraploids with similar or even higher fitness under changing environmental conditions than diploids, suggesting that adaptive changes evolved following WGD.

To further assess whether genomic and phenotypic variation reflects adaptation following whole-genome duplication, and to investigate how diploids and tetraploids respond to selection, which remains uncharacterized, we combined whole-genome sequence data and transplant experiments of diploid and autotetraploid populations sampled at low and high elevation in a factorial design. Tetraploids showed higher non-synonymous genomic variation, consistent with relaxed purifying selection, and a higher proportion of candidate loci associated with adaptation to elevation than diploids. Diploids indeed presented considerable plasticity maintaining fitness homeostasis across elevation, whereas tetraploids showed genetically based fitness variation and phenotypic signatures of adaptation to elevation, suggesting that genomic variation accumulated after WGD enhanced the adaptive potential of tetraploids under environmental changes.

Together, these findings highlight the important role of post-WGD evolution in driving phenotypic and transcriptomic changes. This suggests that WGD is not an inherent driver of adaptation per se, but rather promotes reproductive isolation from progenitors and, supporting the accumulation of high genomic variation, further promotes response to selection in tetraploids that can hence adaptively diverge from diploids.



Evidence of lineage-specific rediploidisation after whole genome duplication in vertebrate ancestor

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MEETING ROOM 118+119, August 18, 2025, 2:00 PM - 5:00 PM

Background: All vertebrate genomes are impacted by multiple rounds of ancestral polyploidisation that have shaped their structure, organisation, and function. Genes arising from these events, termed ohnologs, amount to 20-35% of the human genome and have played a major role in the evolution of vertebrate complexity, development, and susceptibility to genetic disease.

Following whole genome duplication (WGD), chromosomes follow tetrasomic inheritance patterns whereby recombination between homeologous chromosomes is random and equally likely, preventing the divergence of duplicate gene sequences. Thus, any individual gene cannot be considered 'duplicated' until recombination is suppressed. Chromosomes eventually revert to a diploid state in a gradual and asynchronous process termed delayed rediploidisation. Rediploidisation proceeds over a timescale of millions of years and, when overlapping with speciation events, can result in a large number of lineage-specific duplicates, potentially giving rise to unique biological functions.

Previous work found no evidence of delayed rediploidisation after 1R in gnathostomes (jawed) and cyclostomes (jawless vertebrates) and only to a limited extent after the cyclostome-specific genome triplication. However, this work did not account for the GC bias observed in cyclostome genomes, which is well known to cause artefacts in phylogenetic analysis.

Materials and methods: In this study, we re-annotate hox clusters and hox-flanking genes in two hagfish and three lamprey genomes. Using these together with hox clusters from five gnathostome genomes, we build phylogenetic trees based on hox cluster

alignments and classify them according to their rediploidisation timing relative to the cyclostome-gnathostome split. By varying the number of composition vectors in our phylogenetic models, we investigate the effect of composition bias on tree topology. Furthermore, we identify other genomic regions that experienced delayed rediploidisation using a novel macro-synteny-based approach.

Results: When accounting for composition bias, our analysis shows evidence of delayed rediploidisation in hox cluster regions following both 1R and the cyclostome-specific genome triplication. These observations are consistent regardless of technical differences in methodology, such as alignment method or choice of outgroup. Furthermore, we identify other 1R ohnologs and classify them based on divergence time. When mapped onto cyclostome and gnathostome genomes, these genes form distinct pre- and post-speciation clusters on most vertebrate chromosomes, suggesting a widespread impact of delayed rediploidisation on vertebrate genomes.

Conclusions: We show evidence of extensive lineage-specific rediploidisation in cyclostomes and gnathostomes following the vertebrate 1R while highlighting the importance of correctly modelling compositional heterogeneity in these lineages. The mosaic of shared and lineage-specific gene duplications across cyclostome and gnathostome chromosomes suggests a large capacity for lineage-specific adaptations, which may have contributed substantially to the unique biological innovations of each lineage. Our work has major implications for our understanding of vertebrate genome evolution and adaptation following early vertebrate whole genome duplication events.



Evolution of Gene Content and Expression Following Various Whole Genome Duplication Events in Vertebrates

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MEETING ROOM 118+119, August 18, 2025, 2:00 PM - 5:00 PM

A fundamental question in biology is to better understand the molecular bases of phenotypic evolution. Gene and genome duplications, followed by structural and functional rearrangements, are believed to underlie many molecular and phenotypic differences across species, probably contributing to the emergence or modification of tissues and cell types over evolution. Vertebrates offer a unique opportunity to study this phenomenon as they underwent several Whole Genome Duplication events (WGD) at different timescales, providing new raw genetic material for evolutionary forces to act on. Notably, these WGDs have since been followed by extensive genomic and functional reshuffling aimed at reducing redundancy through a process known as rediploidization, likely facilitating the diversification of species, organs, and cell types. In addition, vertebrates exhibit an outstanding phenotypic diversity. In this study, we use large-scale comparative genomics and transcriptomics to explore the evolution of gene content and expression after various WGDs at different timescales across vertebrates. We focused on key WGDs such as the jawed-vertebrate (2R), the teleost-specific (Ts3R), the salmonid-specific (Ss4R) and the carp-specific (Cs4R) ones that occurred approximately 500 mya, 300 mya, 100 mya and 20 mya, respectively. Overall, our work dissected rediploidization processes at both structural and expression levels. Notably, we modeled gene loss trajectories across 160 vertebrate species following WGD events and showed that in addition to the occurrence of WGDs, the rediploidization phenomenon plays a central role in the gene content of extant genomes. We also demonstrated that rediploidization does not occur linearly over evolutionary time, as gene loss is initially massive following WGDs and gradually diminishes, stabilizing at a 'canonical' number of genes in vertebrates. We showed that the initial wave of rediploidization,

driven by extensive gene loss, accelerates genome divergence by proceeding in a lineage-specific manner, thereby reinforcing the role of WGDs as catalysts of evolutionary innovation. Cross-species comparisons of RNA-seq data from seven organs across twelve representative species revealed that duplicated genes are more tissue-specific than singletons. Moreover, the degree of tissue specificity increases with the number of gene copies, confirming that tissue specialization tends to follow gene duplication in fish, whether they underwent ancient or more recent WGDs. However, we also observed that duplicates in recently duplicated genomes are less tissue-specific than those in anciently duplicated genomes, suggesting that tissue specialization generally occurs progressively after genome duplication. To explain this pattern, we proposed and confirmed two non-mutually exclusive scenarios. First, we found that the enhanced tissue specificity observed in anciently duplicated genomes is partly due to the preferential loss of broadly expressed duplicates, leading to the retention of more tissue-specific ones. Second, we observed that differences in tissue specificity are more pronounced among duplicates from anciently duplicated genomes, suggesting that these genes have had more time to accumulate regulatory mutations and develop divergent expression patterns, resulting in greater specialization compared to their paralogs. In conclusion, the results of this study demonstrate the substantial impact of WGDs and subsequent rediploidization processes on gene content and expression in extant vertebrate genomes, likely contributing to the emergence and diversification of tissues and cell types over evolution. We are now applying recently developed cell-focused technologies across species and fast-evolving organs, such as the gonads, to further investigate the role of gene and genome duplications in organ and cell type diversification across species.



Evolution of Regulatory Elements Following Autopolyploidization in Salmonid Fish

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MEETING ROOM 118+119, August 18, 2025, 2:00 PM - 5:00 PM

Background: Whole genome duplication (WGD) is a widespread phenomenon in plant and animal evolution and has been associated with phenotypic diversification and evolutionary innovation. Approximately 100 million years ago, the common ancestor of salmonids experienced a lineage-specific WGD by autopolyploidization, with a substantial proportion of the duplicated genome still retained. This makes salmonids an ideal vertebrate model for studying the impacts of autopolyploidization on genome and regulatory evolution. Beyond gene expression, understanding the evolution of regulatory elements which govern the spatial and temporal patterns of gene activity is crucial to translate the phenotypic outcomes of WGD. In this study, we leverage the extensive functional annotation resources generated by the European AQUA-FAANG project, including over 600 million ATAC-Seq and 4,000 million ChIP-Seq reads, to investigate the fate of duplicated regulatory elements in the genomes of Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). Our primary objective was to assess the conservation and evolution of regulatory element activity across ontogeny and link these patterns to the salmonid-specific WGD event.

Materials and Methods: We generated duplicate-aware whole genome alignments of Atlantic salmon and rainbow trout using Cactus, enabling the accurate alignment of duplicated syntenic regions. Northern pike (*Esox lucius*) was included as an outgroup to infer the ancestral genome state prior to WGD. High-confidence ATAC-Seq peaks from multiple embryonic stages and six adult tissues at two stages of sexual maturation were intersected with the alignments to assess regulatory element activity. We then investigated the coupling between sequence conservation and regulatory element activity across open chromatin regions in duplicated segments. Duplicated promoter and enhancer conservation across developmental stages was systematically analyzed to identify evolutionary constraints.

Furthermore, conserved non-coding elements (CNEs) of varying evolutionary ages were overlapped with identified promoter and enhancer elements to understand conservation of genome regulation across taxa. Lastly, we compared regulatory element conservation between genomic regions with early and late rediploidization histories to infer post-WGD evolutionary dynamics.

Results: Our genome alignments captured a high proportion of duplicated and orthologous sequences across Atlantic salmon, rainbow trout, and northern pike, validating the robustness of our approach. We observed that the proportion of active enhancers retained across duplicated regions increased through embryogenesis, peaking during late somitogenesis. In adult tissues, conservation was highest in the brain and lowest in the testis, with similar trends seen in promoter elements. CNEs of the deepest evolutionary origin were significantly enriched during late embryonic development in ontogeny and in the brain, highlighting the role of ancient regulatory elements in shaping genome function. Our findings highlight the utility of synteny aware genome alignments in elucidating the evolutionary dynamics of regulatory elements post-WGD. The outcomes also broadly align with the developmental hourglass model, demonstrating that regulatory conservation is strongest during the phylotypic stage, extending previous observations into the context of whole genome duplication.

Conclusion: This study provides a detailed functional annotation of two salmonid species and offers a comprehensive analysis of regulatory element evolution following autopolyploidization. Our findings enhance our understanding of how regulatory element activity is linked to ontogeny following WGD, offering important insights into vertebrate genome evolution.



Is there an advantage to polyploidy in animals? *C. elegans* neotetraploids resist severe cold by escaping cold induced death as gravid adults.

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MEETING ROOM 118+119, August 18, 2025, 2:00 PM - 5:00 PM

Gene duplications play a key evolutionary role by providing raw material for functional innovation. Whole Genome Duplication (WGD), or polyploidisation, is a particular case of duplication in which the entire genetic sequence is repeated within the nucleus. In plants, WGD is recognised as a major evolutionary force, and is linked to speciation and the ability to resist periods of stress and of environmental upheaval.

In animals, examples of current polyploid species are rarer, although several ancient WGD events are well documented: for instance, two rounds of WGD occurred during early vertebrate evolution. These events are typically followed by gene loss and rediploidisation, processes that reshape genomes and influence evolutionary trajectories. However, the reasons behind the successful establishment of polyploid animal species remain unclear. While WGD can arise in response to stress, a debated hypothesis suggests that polyploidy may confer selective advantages under stressful conditions, potentially outweighing early fitness costs such as reduced fertility.

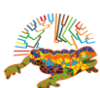
Our aim is to investigate the immediate physiological and stress-related consequences of polyploidy and its potential adaptive value in animals. We are using *Caenorhabditis elegans*, where tetraploidy can be experimentally induced via transient knockdown of *rec-8*, a component of the cohesin complex.

Our results show that while tetraploidy reduces fitness, under regular conditions,

marked by lower fertility and reduced lifespan, tetraploid animals exhibit increased resistance under specific stress conditions, related to temperature changes. While tetraploids exhibit similar pathogen resistance, their response to heat stress is slightly increased. Remarkably, when exposed to severe cold stress followed by recovery, gravid neotetraploid animals massively escape cold-induced death. Additionally, upon cold stress recovery, neotetraploids produce more progeny of comparable quality, suggesting a potential adaptive value.

We investigated whether established mechanisms linked to cold-induced death in gravid diploids operate differently in tetraploids. Previous studies had linked cold-induced death with intestine-to-gonad lipid relocalisation, and to the activation of a cold-triggered genetic death program involving the transcription factor *zip-10*. Our lipid localisation analysis (presented in another poster from the lab) suggests that improved cold survival in tetraploids is not due to defective lipid trafficking. We also found reduced induction of the *zip-10* mediated death program in cold shocked tetraploids although this could only partially account for their improved survival.

Overall, our findings suggest that polyploidy may provide advantages under extreme cold stress and point to novel mechanisms underlying tetraploid resilience to cold-induced death.



Structural variation and dysploidy shape drought responsive gene landscapes in *Nicotiana* sect. *Suaveolentes*

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Whole genome duplication (WGD) provides genetic redundancy that can foster novel adaptations, yet its aftermath, marked by structural variation (SV) and descending dysploidy, remains underexplored in adaptive radiations. These rearrangements reconfigure chromosome architecture, linkage associations, and recombination patterns, potentially clustering or dispersing environment responsive loci. Darlington's chromosome condensation hypothesis proposes that progressive dysploidy physically links co adaptive genes into advantageous blocks, enhancing resilience under stress. To test this idea, we focused on *Nicotiana* section *Suaveolentes*, an allotetraploid clade that arose ca. 6 Mya and subsequently lost chromosomes in a dysploid series ($n = 24 - 15$), coincident with an explosive radiation across Australia. Remarkably, many low count taxa now thrive in the continent's most arid interiors despite retaining thin, herbaceous leaves. Here, we assessed whether drought responsive genes in arid versus mesic lineages are preferentially shielded from structural variants or, instead, concentrated within rearranged genomic regions. For this purpose, we selected two species pairs: the arid adapted *N. velutina* ($n = 16$) vs. its mesic sister *N. gossei* ($n = 18$), and the arid adapted *N. truncata* ($n = 18$) vs. mesic *N. excelsior* ($n = 20$). We generated high quality draft assemblies for both arid taxa: *N. velutina* assembled into 395 contigs (2.71 Gb; N50 = 132 Mb, L90 = 21), and *N. truncata* assembled into 1004 contigs (3.15 Gb; N50 = 122 Mb, L90 = 26). In parallel, we conducted drought experiments on both species pairs: plants were either well watered or subjected to ten days without water then rehydrated 24 h before sampling. Leaf and root tissues

were harvested for RNA seq and analyzed for differential expression (DE). We identified 1 268 and 782 drought responsive genes in leaves and roots of *N. velutina*, and 917 and 1 218 in *N. gossei*. In the second pair, *N. excelsior* yielded 955 leaf and 705 root DE genes, while *N. truncata* showed 1 899 leaf and 1 262 root DE genes. Independently, long reads from each mesic species were aligned to its arid sister's genome to discover SVs, and after excluding ambiguous breakends, we retained over 400 000 high confidence SV events per pair, predominantly deletions and insertions with a minor fraction of inversions. Our key analyses compared the proportion of drought responsive genes that overlap SV regions versus the genomic background. In the *velutina*–*gossei* pair, the arid *N. velutina* showed a significant depletion of drought genes in SV regions (85.3 % overlap vs. 87.8 % background; $p = 0.0082$), consistent with insulation of key loci from rearrangement. Its mesic sister *N. gossei* was neutral (86.5 % vs. 87.8 %; $p = 0.223$). In the *truncata*–*excelsior* pair, mesic *N. excelsior* leaves exhibited enrichment of DE genes in SVs (92.6 % vs. 90.1 %; $p = 0.0076$); arid *N. truncata* leaves were neutral (90.9 % vs. 90.1 %; $p = 0.199$). Across all four species, inversions accounted for > 90 % of these overlaps, but only ca. 20 % of DE genes lay exclusively within inversion intervals. These results offer an empirical validation of Darlington's chromosome condensation hypothesis in a post WGD plant radiation. They reveal that descending dysploidy and SV can either insulate or cluster environment responsive genes in a lineage and tissue specific manner, shaping the genomic architecture that underpins rapid ecological adaptation and speciation in *Nicotiana* section *Suaveolentes*.



The genome evolution of young autopolyploids and their rediploidization

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MEETING ROOM 118+119, August 18, 2025, 2:00 PM - 5:00 PM

Whole genome duplication (WGD) and subsequent rediploidization were ancient, rare, and fundamental evolutionary events that shaped the genomes in early vertebrates. But the dynamics and patterns of these ancient rediploidizations are difficult to investigate. A group of young polyploid fish adapted to living at high altitudes, the snow carp (subfamily Schizothoracine), provides the opportunity to study recent repeated evolution of autopolyploidization that is then followed by re-diploidization. Genomic data from all eleven snow carp genera demonstrate that they are autopolyploid, including tetraploids, hexaploids and rare icosaploids (20n). Using improved bioinformatic approaches, we generated a fully phased chromosome-level genome for *S. younghusbandi* (4n = 90), one of the most specialized snow carp. Comparative genomic analyses identified five unbalanced chromosomal fusions between subgenomes. Further studies of

meiotic karyotypes and sequence divergence suggest only those fused chromosomes evolved disomic inheritance (rediploidization), while the unfused chromosomes remain tetrasomic. Rediploidization was initiated around the fusion points, gradually expanding outwards towards chromosomal arms. We detected signals of biased gene losses on the fused chromosomes, suggesting subgenome dominance driven by chromosomal fusions. Genes with divergent expression on the rediploidized regions, such as *GPX4*, have likely contributed to the adaptation to the extreme conditions of the Qinghai-Tibetan Plateau. We show that all snow carp lineages shared a specific fourth round of WGD (ScR4), followed by lineage-specific rediploidization. Our analyses of the haplotype-resolved snow carp genome highlight the importance of chromosomal fusions in the early evolution of young vertebrate autopolyploids.





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S14 - Evolution at species range margins



A systematic review of hybridization dynamics in frogs: Associations with breeding patterns and courtship behavior

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POSTER SESSION 1 - P01.182, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Hybridization can influence evolutionary trajectories across the tree of life, yet the role of specific reproductive strategies in facilitating or constraining hybridization within animal groups remains underexplored. For instance, frogs exhibit two distinct temporal breeding patterns: explosive breeding, with brief, high-density aggregations and minimal mate selection; and prolonged breeding, with extended reproduction involving mate choice and, at times, elaborate courtship behaviors that may prevent interbreeding. Hybridization is assumed to be more common in explosive breeders without courtship, but the absence of a systematic review limits our understanding of its dynamics in this diverse and threatened order. We conducted a systematic literature review to assess the distribution of hybridization across frog families and genera, and to determine (1) whether hybridization is more frequent in explosive breeders, and (2) whether these species consistently lack courtship compared to prolonged breeders, potentially facilitating interbreeding and hybridization.

Materials and methods: Following the PRISMA protocol, we searched Google Scholar and Web of Science for articles published between 1950 and 2024 using keywords such as 'Anura', 'Hybridization', 'Introgression', 'Explosive breeding', 'Prolonged breeding', and 'Courtship'. We first removed duplicates and screened titles and abstracts to exclude irrelevant studies. We then assessed the remaining full-text articles for eligibility, excluding those that were inaccessible. Additionally, we identified further records through citation searching and excluded those that did not meet eligibility requirements according to PRISMA. We visualized the data and performed Fisher's exact tests to assess the association between courtship and breeding patterns in known hybridization cases in frogs.

Results: We identified 1,801 articles in Google Scholar and Web of Science, and 92 additional articles via citation searching. After initial

screening, 166 articles qualified for full-text review, with 129 meeting inclusion criteria for the final analysis. We identified 88 cases of frog hybridization in 36 genera across 15 families. Of all cases, 50% involved explosive breeders, predominantly in the true toads (Bufonidae), 28% involved prolonged breeders, predominantly within the true tree frogs (Hylidae), and 9% involved one explosive and one prolonged breeder. Breeding patterns were unknown for species in 13% of the cases. Among explosive breeders, courtship was undocumented in 43 cases of hybridization and documented in one. In prolonged breeders, 21 cases lacked documented courtship, while four exhibited courtship behavior. The association between courtship and reproductive patterns in hybridization cases was marginally non-significant, with prolonged breeders tending to exhibit a higher proportion of documented courtships compared to explosive breeders.

Conclusions: Our review indicates that hybridization is widespread in frogs, but the high prevalence in two well-studied families suggests uneven research focus, potentially biasing conclusions about its overall frequency. We corroborated that hybridization is more frequent in explosive breeders, but its occurrence in prolonged breeders, in which courtship was more frequently documented, was substantial enough to challenge the assumption that mate selection and courtship are enough to reinforce species boundaries. Our data suggests that isolation in frogs is more complex than previously thought, with alternative reproductive tactics, such as satellite males, potentially contributing to hybridization. This study underscores the role of reproductive behaviors in shaping species boundaries and evolution, emphasizing the need for integrative research in behavioral ecology and evolutionary genetics to clarify reproductive barrier dynamics, particularly in diverse and vulnerable taxa like frogs.



Adaptive patterns of anti-predator escape behavior in a globally introduced bird species

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POSTER SESSION 1 - **P01.160**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Introduced species can represent quasi-experimental, anthropogenic case studies of both ecological and evolutionary principles. When these species are firmly established, competitive interactions between native and introduced species interactions, including foraging, spacing, and breeding competition, may become costs of such species invasions. In turn, genetic and/or plasticity-driven changes in behavior and morphology could also take place in the invading species with increasing introduction lag (time since the onset of introduction). Critically, however, introduction lag is difficult to study in any single non-native population without long-term observations, and, instead, it requires geographically repeated measures of the focal response variables across invasive populations that were introduced at different times. Here we tested a priori predictors of predator-avoidance behaviors through the flight initiation distance (FID) assay of a widely distributed invasive bird species, the common myna *Acridotheres*

tristis. The species was extensively and consistently sampled throughout most of its independently introduced ranges across all hemispheres. Critically, FID increased with greater introduction lag. We also detected additional functional patterns in that FID increased towards the rural range within a continuous metric of urban-rural gradient and also at shorter distances from the Equator. Any robust study of FID must also include proximate predictors as well and, accordingly, we found that FID increased with greater starting distance, with lower immediate human density, with flighted over walking escape responses, and at lower heights of a bird's perch above ground but was unrelated to myna group size. Respectively, these factors are informative about the sensory cues triggering anti-predator behaviors in invasive mynas and imply an adaptive set of patterns of anti-predator responses in the introduced ranges of this species.



An empirical test of Baker's law: Dispersal favors increased rates of self-fertilization

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POSTER SESSION 1 - P01.169, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Baker's law is the observation that recently dispersed populations are more likely to be self-fertilizing than populations at the range core. The explanatory hypothesis is that dispersal favors self-fertilization due to reproductive assurance. *Caenorhabditis elegans* nematodes reproduce via either self-fertilization or outcrossing and frequently disperse in small numbers to new bacterial food sources. While *C. elegans* males facilitate outcrossing, males and outcrossing are rare in natural *C. elegans* populations. Here, we use experimental evolution to test if frequent dispersal selects for the invasion of self-fertilization into predominantly outcrossing populations. *C. elegans* dispersal often occurs in the dauer alternative life stage. Therefore, we tested

the effects of dispersal on rates of self-fertilization in populations exposed to dauer-inducing conditions and populations maintained under standard lab conditions. Overall, we found that populations required to disperse to new food sources rapidly evolved substantially elevated rates of self-fertilization compared to populations that were not required to disperse in both dauer and non-dauer populations. Our results demonstrate that frequent dispersal can readily favor the evolution of increased selfing rates in *C. elegans* populations, regardless of life stage. These data provide a potential mechanism to explain the dearth of outcrossing in natural populations of *C. elegans*.



Behavioral isolation between two color morphs of the Mourning Wheatear (*Oenanthe lugens*)

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POSTER SESSION 1 - P01.174, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Closely related populations may provide insight into the mechanisms of the early stages of the speciation process. In birds, prezygotic isolation plays a major role in reproductive divergence, usually through behavioral interactions. The Mourning Wheatear (*Oenanthe lugens*) is a territorial insectivore found in arid rocky habitats adjacent to the Saharo-Sindian desert belt. Within the geographical range of the nominate subspecies (*O. l. lugens*) there is a distinct population of a dark morph, restricted to the black lava deserts of northeast Jordan and southern Syria. This population presents a distinct black plumage yet does not show genetic divergence from the typical pale morph. This raises questions about the extent of reproductive isolation between the two morphs and the taxonomic status of the dark morph.

In this study we used behavioral experiments and genetic data in order to examine the reproductive isolation between these morphs. Behavioral assays using dummies tested whether pale-morph territorial

individuals respond differently to their own versus the dark morph. Genetic data was used for phylogenetic reconstruction and population differentiation analysis. Overall behavioral responses did not differ significantly between the morphs, however, pale-morph individuals showed a slightly stronger behavioral reaction to their own morph, suggesting a weak preference for familiar traits. Genetic analyses corroborate the lack of phylogenetic differentiation between the morphs, with 98% of genetic variation occurring within populations, indicating substantial gene flow. These findings suggest that the two morphs may represent populations in the early stages of divergence. While behavioral tendencies hint at emerging reproductive barriers, isolation remains incomplete, and ongoing hybridization is highly likely. Therefore, this study does not justify recognizing the dark morph as a distinct taxon. Further examination of phenotypic and genetic similarities and differences will enhance our understanding of the speciation process of these two morphs.



Common Origins, Divergent Paths: Gonadal Development in the Domestic Chicken and the Zebra Finch in the Context of Avian Evolution

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POSTER SESSION 1 - P01.183, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The domestic chicken (*Gallus gallus domesticus*) and the zebra finch (*Taeniopygia guttata*) are representatives of different evolutionary lines. The phylogenetic lineages leading to domestic chicken (Galloanserae) and zebra finch (Neoaves) diverged in the Cretaceous 71 million years ago. Given the deep evolutionary split between Galloanserae and Neoaves, examining gonadal development in these two species allows us to identify which aspects of sex differentiation are conserved across birds, and which may have evolved uniquely within each lineage.

Eggs were collected on the day they were laid and incubated at 37.5 °C and 70% humidity. Embryos dissected from eggs were staged according to Hamburger-Hamilton tables, fixed in Bouin's solution and embedded in paraffin. We performed histological staining using Dubreuil's modification and to enhance our analysis, we used immunofluorescence with the use of antibodies against factors involved in sex determination.

Our results indicated that asymmetry in gonads is visible from the beginning their primordia formation. The left gonadal ridge is thicker than the right even before the PGCs (primordial germ cells) had settled. After PGCs had settled, we observed that in the zebra finch the left gonadal ridge exhibited a significantly higher number of PGCs than the

right, further enhancing the asymmetry. The ovarian development followed a comparable trajectory in both species, marked by the growth of the cortex and a reduction of the medulla. Notably, asymmetry was evident, as the left ovary had a more prominent cortex, while the right ovary remained underdeveloped. Testis differentiation followed a uniform pattern, involving cortical reduction and medullary expansion. Rapid growth of the right testis minimized asymmetry between both male gonads. Interestingly, in zebra finches, the left testis in some specimens showed disrupted structure, with a hermaphroditic appearance combining seminiferous tubules and ovarian-like cortex.

Our study demonstrates that while the domestic chicken and zebra finch share common early mechanisms of gonadal differentiation, species-specific differences emerge from the initial stages of development and persist following sexual differentiation. Despite their common use as model species in avian research, the chicken and zebra finch exhibit markedly different gonadal development trajectories. This highlights the importance of comparative studies for understanding both conserved and divergent aspects of reproductive development across bird lineages.



Consequences of range shifts on the genetic diversity of butterflies in Finland

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POSTER SESSION 1 - **P01.168**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Many species are shifting their range to track the most suitable environmental conditions in response to global warming. This strategy can improve the chances of species' long-term persistence. However, colonisation events, lower population densities, and strong selection pressures at the expanding range margin can result in reduced genetic diversity at the local populations. Lower habitat availability and connectivity in the range edge can also restrict the expansion, gene flow and population size, with further effects at the genetic level.

Although the loss of genetic diversity during range shift could be only temporary, a rapid and continuous increase in temperatures could cause range shifts to be too swift to allow for genetic diversity recovery. Selection pressures that might help marginal populations expand can also lose effectiveness if the genetic variability is too low for selection to act on. Such scenario will leave expanding edge populations in a particularly vulnerable position. Alternatively, if enough gene flow is maintained between the range edge and other populations, which can be enhanced by the landscape and habitat connectivity, the genetic diversity may be recovered. There is abundant theory on the interplay of

colonisation effects, selection and gene flow on the genetics of edge populations, and simulation studies showing a reduction in genetic diversity due to range expansion. However, few studies have looked at these patterns and the underlying processes in natural populations across different species.

To test these hypotheses, three range-shifting and two non-range-shifting butterfly species in Finland were chosen as model organisms. For each species, ten individuals from three populations in the expanding edge of the distribution range and three in the core of the distribution were sampled for full genome analyses. Each individual was sequenced with a low-coverage whole-genome resequencing approach and will be used to estimate population genetic diversity. We will also look for signs of selection affecting the populations of the range margin. We expect to observe lower genetic diversity in expanding populations than in core populations, and to find stronger differences in species that have expanded northwards over the last decades. Preliminary data from the genetic analyses will be presented here. In future, we aim to link the genetic diversity estimates to the habitat availability and connectivity within the real landscapes.



Dispersal evolution can only rescue a limited set of species from climate change

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POSTER SESSION 1 - P01.161, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Anthropogenic climate change threatens biodiversity on Earth. In response, species can adapt evolutionarily to changing environments or shift their ranges via dispersal. However, dispersal itself can evolve on ecological timescales. We explore theoretically how dispersal evolution modulates the response of metacommunities to climate change. We find that this response depends on the environmental conditions prior to climate change. Variable environments harbour few, dispersive species that are likely to survive climate change by shifting their ranges with

their evolved dispersal abilities. Stable environments house many, less dispersive species. Their survival during climate change is less likely, as they can evolve robust, low dispersal traits that prevent range shifts. We identify a limited set of scenarios in which contemporary dispersal evolution can rescue species from climate change, highlighting the importance of species' evolutionary histories and evolutionary rates, as determined by their genotype-phenotype maps, for their responses to rapid environmental change.



Divergence at the edge: genomic signatures of adaptation in marginal populations of the European mole (*Talpa europaea*)

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POSTER SESSION 1 - **P01.184**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Moles exhibit unique adaptations to a subterranean lifestyle, and their biology and distribution are highly constrained by soil characteristics. Most known species are restricted to relatively narrow regions in the Mediterranean peninsulas and the Middle East, suggesting that geographic isolation and Plio-Pleistocene climatic fluctuations have played a primary role in the speciation processes within the genus. In contrast, the European mole (*Talpa europaea*), unlike most other *Talpa* species, has an unusually wide distribution across Europe. At the southern edge of *T. europaea* range, genetically distinct populations exist in Italy, an area less suitable for the species, where interspecific competition with two other species well adapted to Mediterranean climatic conditions may occur. According to previous studies based on mitochondrial DNA (mtDNA), these Italian populations have been diverging for a long time and remained isolated within the Italian Peninsula even during interglacial phases. As such, the Italian populations represent a valuable system for studying the evolution of this subterranean mammal at the geographic and ecological margins of its range. These data can also provide novel insights into the speciation mechanisms that have shaped the entire genus.

Using a genome-wide SNP dataset, we investigated population structure, historical demography, and signals of local adaptation in *T. europaea*, with a particular focus on this marginal Italian population. Our results confirm the existence of two major genetic lineages, European and Italian, consistent with previous mtDNA analyses. Notably,

northeastern Italy harbors a highly diverse population belonging to the European lineage, possibly reflecting the occurrence of past secondary contact.

Within the Italian lineage, we detected strong genetic substructure, suggesting that environmental heterogeneity across the peninsula, along with potential interspecific competition with other *Talpa* species endemic to the region, has driven further diversification.

Demographic reconstructions revealed that the European lineage underwent post-glacial expansion, while the Italian lineage experienced earlier growth during colder Pleistocene phases. Despite evidence of demographic expansion and potential historical secondary contact, we found weak signs of gene flow between the two lineages, suggesting ongoing reproductive or ecological isolation. Genome-wide scans for selection identified lineage-specific fixed alleles under positive selection in the Italian lineage, particularly in genes associated with hypoxia response pathways, critical for survival in subterranean environments. These findings point to distinct adaptive pressures operating across the species range, especially at its ecological margins.

Overall, our study highlights how climatic history, population structure, isolation, and local adaptation interact to shape evolutionary trajectories at range margins. *T. europaea* serves as a unique model for exploring the genomic basis of divergence and adaptation in marginal populations, with broader implications for understanding evolutionary dynamics and speciation mechanisms in mole species.



Estimating individual stress along expansion ranges: a telomere based approach

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POSTER SESSION 1 - P01.188, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Telomeres, repetitive sequences that protect chromosome extremities, are progressively eroded with aging processes in many taxa. This erosion accelerates under harsh environmental conditions. Consequently, relative telomere length (RTL) is commonly used in ecology as a proxy for physiological stress.

Population expanding in space usually experience contrasted environmental conditions from the core to the edge of their repartition range. Using RTL in this context can provide insight into the relative oxidative stress experienced by colonizing individuals. We investigate this hypothesis through the case of the Kerguelen Islands colonization by

brown trout (*Salmo trutta*), comparing RTL among individuals across different populations along the expansion range. The originality of our approach is that we sampled multiple locations and time points, with up to four temporal samples per location and multiple individuals per temporal sample. This sampling design enables us to disentangle the effects of population age, individual size, and sampling year on RTL, as well as their interactions.

Our aim is to identify a stress gradient along the expansion front and, ultimately, detect footprints of adaptation to the peculiar conditions generated by expanding metapopulations.



Evolutionary suicide in the secondary contact zone

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POSTER SESSION 1 - P01.186, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Rapid evolution can facilitate persistence in the face of environmental change, or increase the likelihood of coexistence between competing species via character displacement. However, adaptation does not always increase the likelihood of population persistence. In this talk, I will present theoretical extensions demonstrating that secondary contact between recently diverged populations can produce a rare case of evolutionary suicide. The logic behind this result is as follows. When males mate with both con- and heterospecific females, they can reduce population growth in heterospecific populations, through the production of inviable zygotes. However, this misdirected mating effort can have large negative effects on male fitness, producing strong selection

on males to discriminate between con- and heterospecific females. Thus, an evolutionary response is expected in both populations, resulting in less severe reproductive interference. However, if the two interacting populations show different evolvabilities for male discriminative ability, the degree to which males reproductively interfere with heterospecific females becomes asymmetric, with males from the slower evolving population having a larger relative effect on the fecundity of females in the other. If this difference in evolvability is sufficiently large, the species showing the more rapid response to selection can be driven to extinction. I will finish by discussing the generality of this result, and by outlining how this effect could be identified in nature.



From Breakthrough to Breakdown? Genetic Consequences of a Rapid Range Expansion in a Key European Pollinator.

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POSTER SESSION 1 - P01.189, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Rapid range expansions place populations under unique evolutionary pressures that shape their success in novel environments. Connectivity between forming subpopulations can maintain genetic diversity—but is this always preserved? Individuals at the expansion front often act as founders, but how much does their contribution define the genetic structure of resulting populations? We utilise the recent and ongoing range expansion of the Tree Bumblebee (*Bombus hypnorum*) to examine evolutionary dynamics at a species' range margin. First recorded in the UK in 2001, *B. hypnorum* rapidly colonised the UK and Ireland and is now one of the country's most abundant bumblebee pollinators. Using a population genomic dataset of 285 individuals spanning both the spatial (continental Europe, UK) and temporal (2002–2015) dimensions of the expansion,

we investigate origin, structure, and genome-wide divergence across the European population. In line with earlier findings, we show that the UK population was not shaped by a severe founding bottleneck, but we detect elevated inbreeding ten years after colonisation. We find no evidence of ongoing admixture with the continental population, and demographic reconstruction reveals a declining effective population size as the expansion progressed. Although overall divergence from the continental source remains low, we identify a set of divergent loci across the genome, suggesting some adaptive potential has been retained. Our findings demonstrate that range expansion and demographic success do not preclude the emergence of genetic vulnerability—particularly at the shifting frontiers of a species' distribution.



Genomic architecture of genetic differentiation in two hybridizing mussels from the mid-Atlantic Ridge

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POSTER SESSION 1 - P01.185, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The mussels *Bathymodiolus azoricus* and *B. puteoserpentis* serve as keystone, ecosystem engineers across the Mid-Atlantic Ridge, creating complex, rugose habitats that support substantial biodiversity at deep-sea hydrothermal vents. These environments, however, meet criteria for both Ecologically and Biologically Significant Areas (EBSAs) under the Convention on Biological Diversity and the FAO's Vulnerable Marine Ecosystems, necessitating protection from anthropogenic disturbances. Of particular concern is the mining of Seafloor Massive Sulphides (SMS), which threatens to disrupt vent plumbing systems and alter local conditions to which these species are specifically adapted, promoting local extinctions. This study, therefore, aimed to elucidate fine-scale patterns of gene flow between species and populations therein of *Bathymodiolus* mussels across 24 hydrothermal sites, spanning approximately 7,000 km of the Mid-Atlantic Ridge, and to determine whether genomic patterns reflect geographic isolation and local adaptation. We employed double-digest restriction site-associated DNA sequencing (ddRAD) to generate genomic libraries and used the *B. heckeriae* reference genome to identify single nucleotide polymorphisms (SNPs) with STACKSv2. Outlier loci were detected using multiple genome-scan approaches. Population genomic structure was assessed through principal component analysis (PCA) and ADMIXTURE, while demographic inference (ongoing) will be conducted using the ABC DILS method. Migration patterns were inferred using divMigrate and compared with larval dispersal models. Genotype-environment associations were investigated through distance-based redundancy analysis (db-RDA). As expected, the first axis of the PCA (PC1) and ADMIXTURE analysis clearly separated the two species with a few F2 and F3 or backcrosses at

the Broken Spur and Snake Pit vent sites. At the species level, outlier SNPs found by genome scan methods corresponded to species-diagnostic markers primarily responsible for variation along PC1. These were concentrated within several portions of chromosomes, indicating chromosome-specific genomic incompatibility between *B. azoricus* and *B. puteoserpentis* (islands of speciation). Outlier SNPs corresponding to PC2 and those highly correlated with environmental variables revealed subtle population structure in both species, pointing to potential temporal instability of introgression over a four-year period and the arrival of foreign alleles from unsampled stepping-stone vents. This temporal evolution of the mussel genetic structure is mainly due to the local extinction of some source populations associated with the vent instability. Functionally significant outlier SNPs were identified within genes involved in DNA repair mechanisms, adaptation to depth, and diurnal rhythms. Changes in allele frequency of outlier loci were highly correlated with latitude, volcanic composition, and sampling year. Our findings reveal a complex, mosaic hybrid zone that likely includes unsampled vents serving as stepping stones for gene flow. Understanding these fine-scale genomic processes provides critical insights into how *Bathymodiolus* populations may respond to anthropogenic disturbances at local scales. These results contribute essential data for evidence-based policy development and the designation of Regional Environmental Management Plans for these vulnerable deep-sea ecosystems. The hitherto unknown temporal instability observed over just four years highlights the dynamic nature of metapopulations where sink populations can become sources and vice versa, emphasising the need for ongoing monitoring and adaptive management approaches.



Genomic diversity of jaguars at the edge of their range

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POSTER SESSION 1 - P01.190, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The jaguar (*Panthera onca*) population in Argentina decreased by more than 95% throughout the 20th century, restricting this species to an estimate of only 250 animals that live in small isolated groups in the northernmost regions of the country. This situation could imply that the last Argentine jaguar populations are undergoing a process of genomic erosion, which would represent a serious threat to their survival. However, a lack of genomic characterization of these animals prevents us from understanding the health status of these populations in terms of genetic diversity.

In this work we propose, to carry out a genomic characterization of this species from samples of Argentine individuals. For this purpose, we sequenced the complete genomes of 9 Argentinean jaguar samples at high depth (>20X). Additionally, we downloaded whole genome sequencing (WGS) data from 12 individuals from other border countries available in SRA. We used perSVade pipeline to process the short

sequencing reads for all these samples, performing quality checks and reads trimming, followed by the alignment of high quality reads to an improved chromosome-level assembly reference genome obtained from a local sample. The complete set of genome-wide variants was obtained through a joint calling approach using GATK tools. To characterize the variation of the generated data, we carried out genomic diversity analysis in order to obtain per-sample heterozygosity levels. We performed a sample clustering based on >11 million of genome-wide SNVs and applied STRUCTURE in search for signatures of population structure among the Argentine samples and in relation to the other American samples. The sustainability of subsisting jaguar populations in the southernmost limit of their current range requires knowledge about the state of genetic variability levels of extant individuals so as to avoid genetic erosion in these populations.



Genomic Effects of the Successful Post-Glacial Expansion of *Bombina pachypus*

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POSTER SESSION 1 - P01.175, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The population expansion range can be a natural response to climate change. In fact, many populations have extended or changed their ranges after the Last Glacial Maximum period, successfully colonizing newly available habitats. The success or failure of such range expansions depends on several ecological and evolutionary factors such as dispersal limitations, changes in gene flow, founder effects, adaptive capacity to novel environments, and the accumulation of deleterious mutations during the expansion, a phenomenon known as expansion load.

Many studies have increasingly focused on expansion load as a limiting factor in the expansion of populations. It is known that allele frequencies change during a range expansion, which can rise in frequency and become fixed at the front of an expanding range due to genetic drift (allele surfing). While allele surfing can occasionally promote adaptive variants, it more commonly facilitates the spread of deleterious alleles, thereby contributing to the expansion load.

Understanding the consequences caused by these phenomena may help in efforts against invasive species or for designing effective conservation translocation programs. As global climate change forces more populations to shift their ranges to survive, these may experience reduced fitness due to expansion load and loss of genetic diversity. These genetic issues can increase extinction risks, especially when populations face stochastic or catastrophic events.

This work aims to analyse the dynamics of masked and realized components of the expansion load and their fitness effects in a case of successful post-glacial expansion. We used the Apennine yellow-bellied toad

(*Bombina pachypus*), an endangered endemic Italian species that expanded from a glacial refugium in Southern Italy northwards along the peninsula after the Last Glacial Maximum, as a model. To simulate this expansion, we used SLiM to develop forward-in-time genomic simulations applying a spatially continuous model. The simulated landscape was generated using past habitat suitability maps derived from ecological niche models, based on current and historical bioclimatic variables and occurrence records of *Bombina pachypus*. To enhance realism, we incorporated key life history traits of the species (dispersal ability, age at reproduction, lifespan), ecological features (competition, carrying capacity, habitat suitability), and genomic factors (accumulation of deleterious and neutral mutations in coding and non-coding simulated regions based on empirical data).

Our preliminary results suggest that the simulations can plausibly replicate the hypothesized colonization route of *B. pachypus* through the Italian peninsula. Moreover, we observe that edge populations accumulate more realized load than core populations, which tend to harbor more masked load, resulting in a greater fitness decline at the expanding front. However, in some simulation replicates, this expansion load does not prevent the population from continuing to colonize new areas.

We are currently conducting further analyses to understand how expansion load varies across different zones of the population (core, intermediate, and marginal areas), and how individual dispersal capacity and intraspecific competition affect the amount of expansion load, fitness reduction, and the overall success of the geographical expansion.



Gradients of increased reproductive effort and increased leakiness in sex expression with range expansion in the dioecious plant *Mercurialis annua*

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POSTER SESSION 1 - **P01.164**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Population density influences both mating opportunities and resource competition in sessile plants. During a species range expansion, edge populations frequently experience low densities, imposing selective pressures on reproductive traits. Notions surrounding ‘Baker’s Law’ predict an increased capacity for uniparental reproduction such as self-fertilization in recently colonized populations in response to selection for reproductive assurance under low mate availability. Similarly, life-history theory predicts a shift toward enhanced reproductive effort in the low-competition environments of colonizing species. Here, we present clear evidence for both these effects in *Mercurialis annua*, a

dioecious wind-pollinated herb that expanded its range across Europe from the eastern Mediterranean Basin. In a common garden experiment using seeds sampled across the species’ natural range, we found a significant increase in the number of individuals with leaky sex expression toward the range margin. Reproductive effort (reproductive number divided by total biomass) also increased with expansion towards the species’ range margins. Our findings thus demonstrate evidence for evolution of both the sexual system and the life history together, consistent with Baker’s Law and increased investment towards reproduction during a range expansion.



Pan-range SNP phylogeography highlights differing effects of postglacial colonisation history on mitochondrial, autosomal, and sex-linked genome compartments

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POSTER SESSION 1 - P01.172, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

During Pleistocene glaciations the Iberian Peninsula comprised multiple refugia, from which spatial genetic clusters and diversity hotspots developed. The routes and timing of recolonisation from such refugia have shaped the current distribution of genetic diversity in this region and throughout Europe. However, this process affects the various genome compartments (nuclear, mtDNA, sex chromosomes) differently. Characterising these differences can yield important insights into evolutionary processes that occur during recolonisation, as well as informing on general evolutionary dynamics of each compartment. In this study we sampled and sequenced (ddRADseq) 468 individuals of the cold-tolerant Iberian endemic amphibian, *Rana iberica*, from 51 sites across its entire species range. Using multiple phylogeographic analyses, including Approximate Bayesian Computation, we find that (1) the metapopulation of *R. iberica* is divided into

broad northern and southern groups corresponding to alternative colonisation routes from the core refugium; (2) genetic diversity is dominated by strong isolation-by-distance, indicating range expansion via stepping stone colonisation; (3) this stepping stone process had profound effects on sex-linked genetic diversity on the sex chromosomes, suggesting different male vs female dispersal tendencies and highlighting the sorely understudied effects of demographics on sex chromosome evolution; and (4) populations in and around the Sistema Central are highly distinct genetically, suggesting a period of consistent and prolonged isolation. These results contribute to our understanding of how cold-tolerant species respond to glacial cycles and the subsequent post-glacial warming that follows, and show how the demographic processes involved can have important and varying effects on different genome compartments.



Phylogeography of the shrimp *Palaemon longirostris* reveals high connectivity along the Atlantic European coast with a latitudinal gradient in diversity

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POSTER SESSION 1 - P01.187, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

This study aimed to investigate intraspecific genetic differentiation and geographic structure in the estuarine shrimp *Palaemon longirostris* along the Atlantic East Coast. The study encompassed 23 estuaries along the Atlantic East Coast. A total of 324 COI sequences were analyzed, including 314 newly generated sequences and 10 retrieved from GenBank. Haplotype networks (TCS), Bayesian skyline plots (BSPs), and AMOVA were employed to assess population structure and genetic differentiation. Rarefaction analyses addressed potential biases from unequal sample sizes across regions. Genetic diversity metrics, including haplotype and nucleotide diversity, were estimated, and patterns of isolation by distance were evaluated using Mantel tests. Analysis revealed a continuous gradient of haplotypes along the latitudinal axis of the

eastern Atlantic coasts, with a notable increase in private haplotypes in the southern region. Three distinct haplogroups were identified: one associated with North Atlantic waters bordering the Western Iberian Peninsula, the Alboran Sea, and North Africa; another comprising estuaries from the Bay of Biscay, the English Channel, and the North Sea; and a third formed by estuaries of the Irish Sea rivers. The Iberian Peninsula exhibited the highest haplotype diversity, aligning with rarefaction results that indicated saturation at higher sampling efforts. Genetic differentiation data indicate a recent population expansion subsequent to a bottleneck or founder event originating from southern populations, with the Gulf of Cadiz acting as both an ancestral zone and a source for estuaries along the Atlantic East Coast.



Populations on the Edge: Range Dynamics and Conservation of Great Crested Newts Under Global Change

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POSTER SESSION 1 - P01.166, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Great crested newts (*Triturus cristatus*) have declined across their range from years of habitat degradation, fragmentation, and loss. They now face new challenges under climate change which could further exacerbate declines. Edge of range populations in the Scottish Highlands are genetically distinct and ecologically important due to the unique habitats they occupy (such as more acidic ponds with pH as low as 4.9) as well as the multitude of processes which may shape their range boundaries in future, including increased drought frequency from warming

temperatures and land-use changes. The aim of this project is to combine individual process-based modelling in RangeshiftR with population genomics methods from target sequence capture data collected across an environmental gradient in the UK and Europe. We will use this to investigate how the interaction of multiple stressors could affect the future persistence, adaptation and range expansion of *T. cristatus* and provide a scientific basis for conservation management strategies in Scotland.



Reconstructing the Evolutionary History of the Genus *Orestias* (Teleostei: Cyprinodontidae): An Integrative Phylogenomic, Morphological, and Biogeographic Approach across the Central Andes

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POSTER SESSION 1 - P01.181, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The genus *Orestias* (Cyprinodontidae) comprises a diverse radiation of freshwater fishes endemic to high-altitude aquatic ecosystems in the Central Andes, particularly the Titicaca-Desaguadero-Poopó-Salares (TDPS) system and adjacent basins across Peru, Bolivia, and Chile. These fishes inhabit dynamic landscapes shaped by paleolake cycles (e.g., Mataro, Minchin, Tauca), altitudinal gradients, and repeated episodes of hydrological isolation, which are hypothesized to have driven speciation through geographic fragmentation. To explore diversification patterns across the genus, we integrated mitochondrial markers (Cyt b, D-loop), genome-wide SNPs, and 54 morphometric traits. Morphological measurements were obtained from 1250 individuals representing 33 species, including type material and specimens housed in major biological collections. Additionally, extensive field sampling was conducted across more than 85 localities covering the known distribution of the genus. Phylogenetic analyses based on molecular data revealed multiple well-supported clades that correspond to historically proposed complexes such as *agassii*, *cuvieri*, and *gilsoni*. However, contrary to earlier taxonomic classifications, we did not recover the *mulleri* group as a cohesive

clade, suggesting its artificial nature. Hierarchical clustering of morphometric data reinforced these molecular groupings and validated species boundaries by grouping holotypes and syntypes within their respective clusters. Biogeographically, our results support Lake Titicaca as a major center of diversification, particularly for the *cuvieri* and *gilsoni* complexes, while the more widely distributed *agassii* complex emerged as polyphyletic, reflecting historical isolation across fragmented Andean basins. The concordance between morphological, genomic, and spatial data highlights the role of past hydrological connections and disconnections in promoting cryptic speciation and adaptive radiation. This integrative study advances our understanding of evolutionary processes in *Orestias*, refining the systematics of the group and emphasizing the importance of landscape history in shaping vertebrate diversity in one of South America's most vulnerable high-altitude ecosystems.

Funding: This work was supported by the Agencia Nacional de Investigación y Desarrollo de Chile (ANID) [grant ANID FONDECYT 1250559; 1221214, 3240022 and CHIC ANID FB210018].



Revisiting Classic Theories to Explain Global Patterns of Genetic Diversity

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POSTER SESSION 1 - P01.179, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Genetic diversity is nature's toolkit for survival, the reservoir of genetic variation that fuels evolution, drives adaptation and builds resilience. It underpins species' long-term survival, making it a key metric for conservation, offering insights into vulnerability and adaptive potential. As global change accelerates, understanding what shapes this diversity has never been more urgent. Mapping global patterns of genetic diversity offers critical insight into the link between microevolutionary processes and macroevolutionary patterns, ultimately helping forecast responses to global change for the identification and optimization of conservation priorities.

Over the past decades, several theories have proposed broad-scale expectations for how genetic diversity is distributed across space. The Central-Marginal Hypothesis (CMH) predicts declining diversity toward the edges of a species' range; the Latitudinal Diversity Gradient in Genetic Diversity (LDG) suggests a decrease in diversity with increasing latitude; and the Refugia Hypothesis (RH) posits that genetic patterns today reflect legacies of isolation in glacial refugia. Though frequently cited and explored in local studies, these theories remain largely untested at global scales with standardized population-level data.

Here, we present GenDivRange, a curated dataset compiling genetic diversity metrics

from the growing body of population-level genomic studies across taxa and regions, and use it to reexamine these foundational hypotheses through a macrogenetic framework. By harmonizing genetic diversity metrics from 1,480 studies, we systematically test the support for these foundational hypotheses across taxa and geographic scales.

Our results show consistent support for the CMH and RH across most taxonomic groups, while LDG-GD patterns appear more taxon- and life-history-specific, reflecting greater context dependency. These findings are in agreement with predictions of long-standing population genetic theory but also highlight the importance of scale and history in shaping genetic diversity. Our framework moves macrogenetics from data mining toward theory-driven synthesis and offers a scalable framework for identifying geographic and taxonomic priorities in large-scale conservation planning, shifting the emphasis from isolated case studies to broad taxonomic patterns. This shift is poised to significantly improve the long-term effectiveness and cost-efficiency of conservation strategies in the face of changing climate. The GenDivRange project welcomes the submission of both new and existing data, fostering data sharing and promoting its application in conservation efforts.



Sex ratio in core, edge and refuge populations of *Mercurialis annua*: Home and away

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POSTER SESSION 1 - **P01.176**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The sex ratios of dioecious plants are expected to approximate 1:1 when parental investment is equal across sexes. However, biased ratios frequently occur, either as primary bias through reduced female or male seed production, or via differential germination and mortality patterns. Under environmental stress, female plants typically underperform compared to males due to higher reproductive costs, resulting in male-biased ratios. Nevertheless, plant adaptation may include sex-specific responses to stressors, potentially maintaining balanced sex ratios in locally adapted populations. Conversely, genetic constraints such as sex chromosomes and wind pollination mechanisms have been associated with female-biased ratios, regardless of the environment.

As part of ECORAMA project, we examined adaptation in the dioecious annual plant *Mercurialis annua* L. across its core range (central Europe), range edge (North-Western Europe), and a glaciation refuge (Middle East). Seeds from these three regions were cultivated for one generation under controlled conditions to eliminate maternal effects, before being planted in 10 experimental plots throughout Europe and the Middle East.

We hypothesized that sex ratio would remain equal across regions, while growing away from home inducing male-biased ratios through environmental stress. Specifically, we predicted that refuge populations would show 1:1 sex ratio in both home and away, due to higher genetic diversity, while edge populations would demonstrate male-biased ratios when grown in core and refuge sites due to environmental stress.

We found that sex ratio did not deviate from 1:1 in core and edge populations in their home region. No significant deviations from 1:1 sex ratio were observed in any origin - experimental site combination, except refuge populations in their home region, which displayed a significant male bias. Additionally, female proportions in refuge populations were slightly higher than core or edge populations across all experimental sites.

We conclude that sex ratio in *M. annua* remains largely unaffected by environmental perturbations. As a wind-pollinated annual with sex chromosomes, *M. annua* maintains consistent sex ratios across environments. Geographical range expansion does not alter sex ratios, suggesting that sex ratio cannot serve as an indicator of adaptation.



Speciation between populations at the margins of *Solanum chilense*: Phenotypic differences and transcriptomics of maternal effects in F1 hybrids.

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POSTER SESSION 1 - **P01.173**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Wild tomatoes (*Solanum* section *Lycopersicon*) are a good model system for studying plant demography and local adaptation to abiotic stresses. Adaptation to the local environment is a primary force driving morphological evolution and allopatric speciation. *Solanum chilense* is considered one of the most promising genetic resource for tomato breeding to biotic and abiotic stresses. From northern Chile, *S. chilense* extended its range by colonizing southwards two arid habitats around the Atacama Desert: 1) along the coast ca. 150,000 years ago, and 2) in the highlands of the Andes ca. 30,000 years ago. These marginal populations evolve in allopatry and show footprints of local adaptation, driving divergence at the genetic and phenotypic levels. This system thus constitutes a model of speciation in action between isolated populations at the margins of the species range. We want to investigate the genetic bases of the observed reproductive isolation between these marginal most southern populations (coastal and highland) of *S. chilense*.

First, we document phenotypic and genetic differences between populations. We compare the effect of drought under controlled climatic conditions across

populations of *Solanum chilense*. We use seven parental accessions —LA4107, LA3111, LA1963, LA4330, LA4117A, LA2932, LA2755—from different local habitats. We measure seed germination rate, plant growth parameters, physiological responses, and morphological traits. Our results reveal wide variability within and between accessions, indicating the polygenic and multi-trait nature of drought tolerance and local adaptation in *Solanum chilense*.

We successfully produced both F1 and F2 generations based on various crosses between coastal and highland populations and indeed observed strong incompatibility. We notably observed a pronounced maternal effect in the crosses between coastal and highland populations LA4107× LA4117A. To further validate and decipher the genetic bases of this maternal incompatibility effect, we are conducting an RNA-Seq experiment focusing on early root and stem developmental stages. Our results reveal differentially expressed genes likely responsible for developmental differences between the reciprocal crosses, and we use genomic data to assess whether these genes also exhibit signatures of local adaptation, such as selective sweeps.



The Evolutionary Path and Divergence of the *Oncocyclus* Irises Species Complex Across the Southern Levant

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POSTER SESSION 1 - **P01.178**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Understanding the mechanisms by which populations evolve and diverge into distinct species is a central question in evolutionary biology. Speciation is a continuous process, with species often existing at various stages of divergence. Despite extensive research on speciation, it was rarely studied in species in the course of speciation, which can offer valuable insights into divergence evolution.

Materials and Methods: This study focuses on eight Israeli *Oncocyclus* Iris species, commonly known as the Royal Irises, which are thought to be in an ongoing speciation process. These species are either endemic or sub-endemic to Israel, with populations that are highly fragmented and distributed across a range of ecological habitats. Our goal was to explore the roles of geographic isolation and ecological differentiation in the divergence process of this evolutionary-young group. We employed restriction-site associated DNA sequencing (RADseq) to reconstruct the phylogeographic history and

to decipher the spatial and ecological evolutionary relationships among populations.

Results: Our phylogenetic analyses were largely consistent with current taxonomic classifications. *Iris bismarckiana* consistently emerged as genetically distinct from all other species. In most analyses, *I. atropurpurea* and *I. mariae* also formed a separate clade. However, several species traditionally defined by morphological traits and distribution could not be genetically differentiated. We found significant correlations between genetic relatedness and both geographic proximity and ecological variables.

Conclusions: These findings indicate that both geographic separation and adaptation to local environmental conditions contribute to genetic divergence within the Royal Irises. This supports the hypothesis that they represent a species complex in the early stages of speciation.



The genotype-phenotype map of repeated seasonal adaptation during range expansion of a butterfly

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POSTER SESSION 1 - **P01.180**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The relationship between genotype and phenotype is a central aspect of evolutionary biology. It determines how phenotypic selection affects genetic variation which may lead to adaptation in natural populations. Examples of repeated phenotypic adaptation in response to similar selection (through parallel or convergent evolution) raises the question if it is typically due to parallel genetic changes? I.e. is the repeated evolution of the same trait value due to selection among allelic variants of the same genes, so called gene reuse, or are there alternative genomic routes leading to the same phenotypic adaptation?

The evolution of photoperiodism for life cycle regulation in insects have led to repeated, and remarkably predictable, adaptive clines across latitudes. The shared selection pressures, due to highly predictable changes in daylengths across latitude and strong selection on the annual number of generations (voltinism), are relatively well understood. However, to what extent this repeated adaptation is due to genetic parallelism and gene reuse is still unknown. We have explored this question by assessing the consistency of the genotype-phenotype map during repeated evolution of photoperiodism for induction of winter dormancy (diapause) in the northern range margin of the Speckled wood butterfly (*Pararge aegeria*). In common garden experiments we demonstrate adaptive variation in the critical daylength for diapause induction (CDL) among latitudinally distinct Swedish populations, while a phylogeographic analysis of whole-

genome sequences suggests that similar CDL:s have evolved repeatedly during at least three independent colonizations of new and still isolated areas (large Baltic islands). A QTL analysis using crosses between two distant populations and a GWAS of nine Swedish populations converged on both Z-linked and autosomal genes in the circadian clock being associated with adaptive variation in CDL. However, independent crosses between several different populations suggest that while Z-linked genetic variation is associated with CDL evolution among populations at the Swedish mainland, it has not been involved in the evolution of CDL on the isolated Baltic island of Gotland. Instead, we show that this island population appears to be fixed for a large deletion in the autosomal clock gene, *timeless*, that when introgressed into a mainland population recovers a large proportion of the Gotland CDL phenotype. Hence, we conclude that the evolution of CDL in the Gotland population appears largely to be due to a structural mutation that does not exist in any of the other populations with a similar CDL. In combination these results suggest that the genotype-phenotype map for repeated adaptation of CDL in *P. aegeria* varies across the species range margin. However, we still find a recurrent pattern of allelic variation at circadian clock genes, including *timeless*, being associated with the evolution of photoperiodism across populations, suggesting a strong element of gene reuse during repeated seasonal adaptation in the northern range margin of *P. aegeria*.



The K-tabase: A Comprehensive Database of Plant Genetic Diversity and Population Structure Statistics for Macrogenetic Analysis.

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POSTER SESSION 1 - P01.170, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Macrogenetics, the study of large-scale patterns and predictors of intraspecific genetic variation across species, can play a significant role in our understanding of evolution and biodiversity. The number of genetic clusters (K) is a commonly reported metric of genetic structure, that provides information about how genetic populations are distributed within and across geographic regions. However, there has been little empirical effort to synthesize the information in 'K' across studies to address global questions, such as how latitude, phytogeographic boundaries, and distance from past refugia influence the degree of genetic diversity and structure within plant taxa. Utilising published datasets, I am curating the "K-tabase", a database containing population structure and genetic diversity metrics for over 2000 plant taxa from across the globe. With the large

taxonomic representation and spatial coverage, I will evaluate how impacts of phylogeny, life history, biogeography, and environments shape global patterns of population structure. I will further test how methodological factors such as spatial scale, sample sizes, genetic marker types, and estimation method impact inference and comparability of spatial genetic structure estimates across studies. The K-tabase will enable mapping of global variation in population structure, identify hotspots and breakpoints where genetic structure patterns overlap across many species, and develop insight on how these hotspots compare to other global partitioning of biogeographic zones and boundaries, providing a useful resource integrating macro-and micro-evolutionary processes for biogeography, conservation, and policy.



Uncovering Hybridization and Species Boundaries in Peripheral Populations of the Great Tit (*Parus major*) Using High-Resolution Genomics

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POSTER SESSION 1 - P01.171, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Hybrid zones at range margins offer unique opportunities to investigate how genetic novelties emerge and persist over evolutionary timescales. Peripheral populations often experience distinct selective pressures and increased genetic drift, facilitating the formation of novel genetic combinations through hybridization—combinations that may drive local adaptation or even the origin of new taxa. In the *Parus major* species complex, which includes four phylogenetic lineages across Eurasia (*bokharensis*, *cinereous*, *minor*, and *major*), secondary contact has occurred since the Pleistocene, resulting in several zones of hybridization. We focus on a proposed three-species contact zone in northeastern Iran involving *bokharensis*, *cinereous*, and *major*, with particular attention to the hypothesized hybrid origin of *Parus major intermedius*. Using high-resolution genomic data, we find evidence of recent introgression between *intermedius* and *bokharensis*; however, *P. m. intermedius* does not conform to the

classical definition of a hybrid species. Instead, it displays unique genetic signatures that point to a complex demographic history. Mitochondrial haplotype networks and phylogenetic tree analyses reveal that *P. m. intermedius* is genetically distinct from central European populations. Moreover, its low mitochondrial diversity and high genetic differentiation suggest that it represents a peripheral population. We also detect ongoing gene flow within the contact zone: *bokharensis* individuals exhibit introgression from *major*, while European *major* populations retain signatures of ongoing introgression from *intermedius*. These introgressed genotypes contribute to the genetic diversity of the *Parus major* complex and may serve as a reservoir for adaptive potential. Our findings shed light on the intricate dynamics of hybridization and introgression, offering valuable insights into speciation and the genetic architecture of species boundaries shaped by both ancient and ongoing evolutionary processes.



Unraveling the evolutionary history of *Orestias agassii*: a phylogeographic study considering its current range.

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POSTER SESSION 1 - P01.163, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The Altiplano system serves as a natural laboratory for studying diversification due to its complex geological and paleoclimatic history. The genus *Orestias* comprises 46 endemic species found in the Altiplano and high-altitude inter-Andean systems in Peru, Bolivia, and Chile. Previous research has mainly focused on morphological analyses; however, unclear species boundaries and inconsistent taxonomic practices have complicated species identification. *Orestias agassii* is the most widely distributed species in this genus, occurring across various hydrographic basins in Chile, Bolivia, and Peru. Consequently, individuals identified as *O. agassii* may actually belong to several different species.

In this study, we conducted a systematic and phylogeographic analysis across the entire distribution range of *O. agassii*. We sampled 65 localities and collected a total of 710 individual specimens. We amplified the mitochondrial markers control region D-loop and Cytochrome b and performed phylogenetic reconstructions using both Bayesian Inference and Maximum Likelihood methods. We assessed genetic diversity through various indices and haplotype networks. Patterns of population structuring were examined using the SAMOVA and Geneland programs. Additionally, we conducted species delimitation using Cytb sequences employing three different methods: ABGD, mPTP, and ASAP.

Genetic structuring analyses revealed a clear pattern of differentiation between the Amazon basin in the Cusco region and the other basins included in our study. This distinction was evident in both haplotypic networks and the topology of the phylogenetic trees. Moreover, all other basins formed a cohesive unit. Phylogenetic analyses and haplotype networks suggested that this pattern is closely related to the hydrography of the Altiplano. Additionally, species delimitation analyses identified five candidate species. These findings are consistent with the phylogenetic and genetic structuring results, which indicate that the Cusco localities are distinctly separate from all others. Meanwhile, the Titicaca, Desaguadero, Beni, Pampa del Tamarugal, Uyuni, and Altiplano basins collectively form a single group.

We conclude that the current distribution of *O. agassii* does not correspond to a single species. Instead, we propose the following candidate species/ lineages: the first comprises the populations from Chile, Bolivia, and Peru (excluding Cusco), which corresponds to *O. agassii*; the second includes three distinct species or lineage for the Amazon basin, incorporating the Cusco localities.

Funding: This work was supported by the Agencia Nacional de Investigación y Desarrollo de Chile (ANID) [grant ANID FONDECYT 1250559, 1221214, 3240022 and CHIC ANID FB210018].



Where we're going, we won't need eyes to see: Distributional range size drivers for subterranean spiders

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POSTER SESSION 1 - P01.162, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Understanding species' range size and why closely related species can have drastically different distributions is a fundamental question in biology. This has implications in ecology, evolutionary biology, biogeography, and species conservation. However, answering this question is challenging due to the multiple inter-related factors that shape species distributions, particularly in continental regions, where unclear ecological and geographic boundaries and long evolutionary histories obscure the main drivers of range size variation.

Island-like ecosystems such as caves, oceanic islands, and mountain tops provide exceptional models for studying species' biogeography and evolution due to their well-defined boundaries and relatively simplified ecosystems. Despite their advantages for research, subterranean habitats remain poorly studied compared to surface ecosystems, largely due to accessibility challenges—a phenomenon often referred to as the Racovitzan impediment. Paradoxically, these habitats, which offer key insights into evolutionary and ecological processes, are also highly vulnerable to human-induced impacts, putting species living there at risk of extinction even before we can fully understand their evolutionary history.

In this study, we aim to better understand the factors influencing distributional range size in cave and subterranean species, using European spiders as a model system.

Materials and Methods: We compiled the most up-to-date database of European cave and subterranean spider distributions to analyze the factors shaping their range sizes. We used Variation Partitioning Analysis to determine the relative influence of phenotypic cave adaptations, historical factors, and potential sampling biases on various range size metrics.

Results: Our results indicate that the most significant determinant of range size in subterranean spiders is the degree of phenotypic adaptation to a subterranean lifestyle, particularly eye reduction and, secondarily, loss of pigmentation, whereas limb elongation had no significant effect. In contrast, historical factors, such as past temperature fluctuations and ice coverage during the Last Glacial Maximum, played a lesser role. Sampling biases had minimal impact on range sizes, but other unaccounted factors and taxonomic relationships also appeared to influence species distributional range.

Conclusions: Adaptation to highly selective subterranean environments leads to specialized traits, with eye loss being one of the most distinctive. Our findings suggest that this trait is also linked to a reduction in species' range size among European subterranean spiders. Those findings will help future evolutionary and conservation studies focused in these often poorly understood ecosystems.



Adaptation to 44 years of climate change across a gradient from niche center to edge

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MEETING ROOM 129+130, August 18, 2025, 2:00 PM - 5:00 PM

Background: Evolution is expected to contribute to the persistence of populations exposed to changing climate, especially at range margins along the warm edge of a species' distribution. However, demonstrations of rapid evolution in wild populations associated with climate change are extremely scarce. Instead, inferences about possible adaptive responses often rely on "space-for-time" approaches, which have assumptions that can be difficult to verify. Resurrection experiments offer a direct method to observe evolutionary changes over time, but these rarely span the periods of several decades over which climatic shifts have occurred. In this study, we combine space-for-time inferences, based on genomic and phenotypic data, with resurrection experiments to investigate the evolution of the annual plant *Lactuca serriola* following 44 years of climate change near its arid range edge in Israel. We ask (1) do populations show signs of adaptation to climate along a gradient from niche center to niche edge? (2) Have populations evolved to changing climate? (3) What are the implications of this for evolution at range margins?

Materials and methods: In 2020 we sampled 21 populations of *L. serriola* across Israel, spanning a steep precipitation gradient from the mesic north to the arid south, where the species reaches its arid range and niche edge. We first used low-coverage whole genome sequencing (n=329) and environmental association analyses to test for genomic signatures of adaptation across this gradient from niche center to edge. Next, seeds from 2020 populations were paired with collections made from the same 21 locations in 1976. Plants from these pairings were grown in a common garden in a greenhouse in Zürich, Switzerland, for two generations, and monitored for phenological transitions. We focused on phenology as it is known from previous work to be involved in climate

adaptation in this species, with advanced flowering associated with populations from warmer and drier climates. We used mixed effects models to test the hypothesis that flowering time is earlier in populations from more arid climates (space-for-time prediction) and in the 2020 vs 1976 cohorts (climate change prediction).

Results: We identified 108 candidate genes with significant associations with Israel's gradient in precipitation of the wettest month. Partial allelic turnover for the majority of these genes (67%) was strongest towards the niche edge. Enrichment analyses revealed selection acted on genes associated with phenology and abiotic stress responses at the niche edge, and biotic interactions (e.g. defense) closer to the niche center. In the common garden experiment, we found that flowering time in the second greenhouse generation was over 6 days earlier in contemporary (2020 cohort) than ancestral plants (1976 cohort). However, contrary to our hypothesis, plants originating from drier populations flowered later than those from wetter populations, and there was no significant interaction between the precipitation gradient and cohort.

Conclusions: Our findings provide direct evidence for rapid adaptation to climate change in a wild species, even at its arid range edge. Hindcasting of the adaptive genetic composition of our populations based on the environmental associations observed in 2020 also suggest that adaptation has occurred at the range edge in the last 44 years. What the limits to this adaptation are, however, remain to be determined. Furthermore, the mismatch between space-for-time predictions and observed evolutionary shifts in phenology over time caution about inferring evolutionary responses to climate change from spatial adaptive variation observed today.



Digestive efficiency and mitochondrial phenotype underlie the rapid evolution of a faster pace-of-life at the margins of a range-expanding species

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MEETING ROOM 129+130, August 18, 2025, 2:00 PM - 5:00 PM

Due to global change, many species are shifting their distribution and encounter new thermal conditions. Evidence is mounting that this may not only cause plastic trait changes, but also rapid evolution of traits and of their thermal plasticity. These traits do not react and evolve to the environment in isolation but they form a correlated set of life-history, physiological and behavioural traits that make up a pace-of-life (POL). The POL concept states that life-history, behavioural and physiological traits covary together as the result of energy trade-offs in allocating resources toward growth, survival and reproduction and thereby align along a fast to slow continuum. Despite that the POL concept has proven to be a powerful predictive framework, to explain patterns of trait macro-evolution among species, much less is known whether it also can predict trait evolution among the populations at the range margins. In addition, an emerging hypothesis suggests that, apart from the food digestive metabolism, the mitochondrial phenotype (efficiency and density) contributes to POL variations across the thermal regimes at the range margins. To deepen this understanding, we investigated the thermal evolution and plasticity of the POL and its underlying mechanisms by making use of the southward range expansion of the damselfly *Ischnura elegans*, from France into warmer regions of Spain. Warmer temperatures generally result in a faster POL. However, unfavourable habitats at the range margins, due to founder effects and low densities, may instead result in a slower POL. This contrast makes

studying a southward range expansion particularly intriguing. Alongside the ancestral French region, we studied two Spanish regions: a 'new edge' region founded 10 years ago (Salamanca) and an 'old edge' region founded 70 years ago (Murcia-Alicante). In a common garden experiment, larvae and adults from all regions were exposed to French and Spanish summer temperatures. Our results confirmed that the old edge region evolved a faster POL than the ancestral region when exposed to Spanish temperatures. As hypothesized, the old edge individuals had higher mitochondrial and digestive efficiencies supporting their evolved fast POL. Additionally, the larvae and adults showed higher mitochondrial densities, further sustaining this pace. In contrast, the new edge region showed maladaptive traits, exhibiting a slower POL than the ancestral region when exposed to Spanish temperatures. Such maladaptive responses may reflect that populations at the new range margin indeed occupy less favourable habitats with lower densities compared to the ancestral region. In addition, these individuals increased their food intake, likely prioritizing energy allocation toward repair mechanisms to cope with thermal stress. In conclusion, our findings highlight that the traditional POL framework, which links food intake to fast POL traits, may require refinement as we show for the first time that the mitochondrial phenotype can be an important driver of rapid evolution of the POL at the range margins.



Eco-evolutionary feedback of thermal adaptation during range expansion

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MEETING ROOM 129+130, August 18, 2025, 2:00 PM - 5:00 PM

Temperature fundamentally drives biological processes across scales—from protein kinetics to species distributions. While molecular studies focus on protein stability and enzyme function, and ecological studies often emphasize emergent traits like fitness or range shifts, these perspectives are rarely integrated. This disconnect limits our understanding of how protein-level thermal adaptation scales up to influence ecological and evolutionary patterns.

We bridge this gap by integrating two mechanistic models of temperature-dependent biological rates into an eco-evolutionary range expansion framework. First, we consider the Protein denaturation based model (Chen et al. 2019), which links organismal growth to the fraction of functional proteins and has been supported by studies across viruses, bacteria, and insects. Second, we apply the Macromolecular rate theory (Hobbs et al. 2013), grounded in transition state theory, which attributes thermal performance curve (TPC) curvature to enthalpic and entropic

changes in enzyme reactions—especially relevant in cold-adapted species.

By embedding these models within a spatially explicit, evolving population model, we investigate how thermodynamic constraints affect birth, death, and density dependence, and thus shape range expansion dynamics. Using simulations, we compare how each model influences expansion speeds, asymmetry in TPCs, and emergent trait distributions across landscapes.

Our results show that mechanistic assumptions at the molecular level critically affect macroecological outcomes. Specifically, different sensitivities to mutations under each model lead to distinct thermal trait evolution patterns, range limits, and biodiversity maintenance mechanisms. We highlight the utility of mutation sensitivity maps in linking molecular adaptation to eco-evolutionary dynamics, emphasizing that a pluralistic, thermodynamically-informed framework is essential for predicting biological responses to global temperature change.



Flowering responses of the woodland strawberry to local climate and reduced precipitation along a European latitudinal gradient

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MEETING ROOM 129+130, August 18, 2025, 2:00 PM - 5:00 PM

Climate change creates novel environmental conditions that plant species must adapt to. Since plants are finely tuned to the seasonality of their environments, shifts in the timing of their phenological activities serve as some of the most compelling evidence of climate change's impact. Thus, understanding how key phenological traits linked to fitness, such as the onset of flowering, respond to novel environments is crucial for assessing the plastic and/or adaptive potential of species to persist in the face of climate change. In this study, we investigated the onset of flowering in *Fragaria vesca* (woodland strawberry; Rosaceae) by translocating genotypes between four sites along a south-north gradient in Europe, encompassing its entire latitudinal distribution range with varying temperatures, precipitation patterns, and photoperiods. At each site, we included a reduced precipitation treatment using rain-out shelters to simulate drought conditions and assess their impact on flowering onset. Our findings revealed that southern and central European genotypes exhibited a delayed

onset of flowering when translocated to the northernmost site. In contrast, no difference among genotypes was found in the onset of flowering when grown in more southerly sites. Additionally, reduced precipitation accelerated flowering across several sites and all genotypes, irrespective of their latitudinal origin. Overall, our results revealed that northern European genotypes showed a greater capacity to adjust their onset of flowering in response to the different photoperiods and temperatures across the latitudinal gradient compared to southern European genotypes, suggesting that they may be more resilient to shifting environmental conditions. Furthermore, the differential response of phenotypic plasticity across genotypes, when translocated at higher versus lower latitudes, highlights the role of photoperiod when assessing a species' ability to cope with climate change.

Keywords: Drought, *Fragaria vesca*, Growing Degree days, Onset of flowering, Photoperiod, Plant phenology.



Genetic variation in plasticity at different environmental scales determines fitness variation within and outside the elevational range margin of *Senecio* daisies.

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MEETING ROOM 129+130, August 18, 2025, 2:00 PM - 5:00 PM

When genotypes show reduced fitness due to the phenotypes they produce in novel environments, genetic variation in plasticity could aide population persistence by reducing the severity of predicted fitness declines. However, we know little about the potential for standing genetic variation in plasticity to determine population persistence beyond current range margins. At four elevations on Mt Etna, we transplanted 40 clones per elevation for each of 19 genotypes of a Sicilian daisy, *Senecio chrysanthemifolius*, and estimated their plasticity and fitness within and native and novel elevations. We show that different

directions and magnitudes of plasticity are favoured across elevations, and that genetic variation in plasticity has different effects on fitness in novel and native environments, as well as within versus among elevations. Different genotypes also show surprisingly different patterns of gene expression variation that affect fitness in response to these native and novel environments. In particular, we show that genetic variation in plasticity that is typically hidden within the native range becomes visible in novel environments, and may be crucial for allowing population persistence when faced with rapid environmental change.



Sexual selection shapes eco-evolutionary dynamics at range margins

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Understanding what processes shape the dynamics of species' geographic range limits is one ongoing central question in ecology and evolution. One potentially key process is sexual selection; yet theory examining how sexual selection could affect eco-evolutionary dynamics in marginal populations is still lacking. In species with separate sexes, range limits could be shaped by limitations in encountering mates at low densities. Sexual selection could therefore modulate mate limitation and thereby extinction-colonisation dynamics at range margins, through evolution of mate encounter ability and/or mate competition traits, and their demographic consequences. Further, as sexual selection has been hypothesised to reduce genetic load, it may play a role in preventing the accumulation of expansion load during species' range expansion and thereby prevent reduction in

fitness at the leading front populations. I will present results from two theoretical models. The first model demonstrates that evolution of species' ranges may be importantly shaped by feedbacks between sexual selection and spatial population demography and dynamics. The second model shows that sexual selection, in interaction with dispersal evolution, may have a considerable, sometimes counterintuitive, role in reducing accumulation of expansion load in front populations. Together, these results underscore the potential role of sexual selection in shaping eco-evolutionary dynamics at range margins, highlighting the need for further theoretical and empirical exploration in this relatively understudied area.



The causes of elevational range limits: insights from population genomic and quantitative genetic approaches

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MEETING ROOM 129+130, August 18, 2025, 2:00 PM - 5:00 PM

Background: Why species are restricted within spatial boundaries is a central question in ecology and evolutionary biology, and it has been studied for over 200 years. With no general answers, the search for the causes of range limits is ongoing. Several possible causes may contribute to range limits, including (but not limited to) depletion of genetic diversity due to small population sizes and genetic drift, and low genetic variances for key adaptive traits. Further, an understudied but potentially important cause may be the genetic non-independence of traits, which can constrain adaptation beyond boundaries. Here, we conducted a comprehensive evaluation of why the evolutionary niche of species is often conserved. We tested this hypothesis on elevational gradients, where environmental change occurs over short geographic distances.

Methods: For the first part, we collected seeds of three high- and three low-elevation Brassicaceae species in the core and both the lower and upper range edges, from two separate mountain transects in different regions of Switzerland. We estimated population-level inbreeding coefficients and genetic differentiation among populations using whole-genome sequencing data. For the second, we chose one transect per species and raised offspring plants of matrilineal lines in the greenhouse under two thermal treatments: warm and control. We measured key growth and leaf traits that diverge across elevation in Brassicaceae and estimated the genetic covariance (G-)matrix for each population and the two growth treatments.

Results: Based on the population genomic approach, we found that populations from elevational range edges did not have reduced genomic diversity nor were they more inbred relative to populations from the core. However, all populations on a transect were genetically differentiated, sometimes to small but always to significant extents. Based on the quantitative genetic approach, we found that G-matrices were relatively stable between treatments and among populations of high-elevation species, though we detected strong differentiation among populations for the low-elevation species. Multivariate genetic variances were lower in high- as compared to low-elevation species, potentially threatening the high-elevation species with lower adaptive potential. When constraining effects of genetic covariances were considered, evolvabilities diminished proportionally for all species and populations, indicating the general role of genetic constraints in reducing the adaptive potential.

Conclusions: Overall, our combined population genomic and quantitative genetic approach yielded deep insights into the causes of elevational ranges in Brassicaceae. First, our results do not support the hypothesis that reduced effective population size is involved – directly or indirectly – in the context of elevational ranges. Second, our findings indicate that the genetic architecture constrains the evolutionary response and contributes to maintaining species' elevational range limits.



The impact of climate change on the adaptive potential of alpine plants

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MEETING ROOM 129+130, August 18, 2025, 2:00 PM - 5:00 PM

A species' adaptive potential is crucial for determining its chances of persistence when facing climate change. Previous studies have suggested that plants can evolve in response to climate change, yet little is known about how alterations in selection regimes under climate change can act to alter adaptive potential. Here, we use a transplant experiment along an elevation gradient, simulating climate warming and changing competitive interactions, to investigate the potential role of selection imposed under climate change in shaping the adaptive potential of three alpine plant species. First, we empirically estimated directional, stabilizing, disruptive and correlational selection acting on leaf morphology under scenarios of gradual warming and changing competitive interactions. Second, we inferred how

changes in selection regimes due to climate warming and changing competitive interactions acted to alter the adaptive potential through their effects on simulated G-matrices. Warming caused shifts from disruptive to non-significant and stabilizing selection for *Plantago alpina* and *Anthyllis vulneraria* ssp. *alpestris*, respectively, which acted to reduce adaptive potential. When *P. alpina* interacted with low instead of high-elevation species, selection shifted from non-significant to stabilizing, further contributing to reducing adaptive potential. Our study highlights that apart from selection imposed by climate warming, altered selection regimes under changing competitive interactions play crucial roles in shaping responses in alpine plants facing climate change, by eroding their adaptive potential.



Winters restrict a climate change-driven butterfly range expansion despite rapid evolution of seasonal timing traits at the range margin

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MEETING ROOM 129+130, August 18, 2025, 2:00 PM - 5:00 PM

Background: Climate change pushes species toward higher latitudes and altitudes, but the proximate drivers of range expansions vary, and it is unclear whether evolution at range margins facilitates climate change-induced range changes. Over the past 20 years, wall brown butterflies (*Lasiommata megera*) have rapidly expanded northward in Sweden in association with climate change, providing the opportunity to test for a role of range margin evolution in this range expansion. A previous, lab-based common garden study identified evolution of winter diapause cues in range margin populations, but what effect does this have under natural conditions and does this trait actually limit the range?

Materials and Methods: We conducted a reciprocal transplant experiment, raising larvae descended from range interior and range margin populations of *L. megera* at sites 1) in the range interior, 2) at the northern range margin, and 3) beyond the current range margin. At the beginning of winter, we measured traits related to seasonal timing (diapause induction and growth rate), and after winter we checked their overwinter survival. With this experiment we both tested for local adaptation in these traits and evaluated to what extent local adaptation influences the on-going, climate-driven range expansion.

Results: Almost all individuals from all populations entered diapause at an appropriate time, despite the previously identified among-population variation in diapause induction thresholds. Caterpillars of northern descent, however, grew faster than those from southern populations, at all field sites. This may be a counter-gradient adaptation to compensate for the short, northern growing seasons, but we found no evidence for pre-winter body mass affecting winter survival. In fact, winter survival was low overall—extremely so at the beyond range site—regardless of population origin, indicating that the primary constraint to range expansion is an inability to adapt to winter conditions.

Conclusions: Although range-expanding *L. megera* show clear local evolution of two traits related to seasonal timing, these putative local adaptations likely do not contribute to range expansion, which is instead limited by winter survival. Thus, despite clear evidence for evolution of multiple traits at an expanding range margin, it is instead the unevolving traits that appear to set the range margin. To predict future range changes, it will therefore be important to distinguish between the traits that evolve during range expansion and those that set the range margin.





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S15 - Evolution in and of diverse genetic systems



Diversity of sex chromosome gene regulation in squamate reptiles

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POSTER SESSION 2 - P02.053, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Differentiation of sex chromosomes is typically accompanied by the cessation of recombination and subsequent gene loss from the degenerated sex chromosome (Y or W), resulting in gene dosage imbalances between the sexes. Classical theory, based largely on studies in model organisms, predicts the evolution of mechanisms to compensate for these imbalances. Dosage compensation - or at least dosage balance in the expression of sex-linked genes - has been well documented in lineages with male heterogamety (XX/XY). In contrast, lineages with female heterogamety (ZZ/ZW) are often

thought to lack global, chromosome-wide regulatory mechanisms for gene dosage, particularly at the transcriptional level. In this study, we combined high-throughput sequencing with comparative bioinformatics to investigate patterns of transcriptional gene dose regulation in nine independently evolved sex chromosome systems of squamate reptiles. Our findings provide novel evidence from non-traditional model organisms, revealing the molecular diversity and evolutionary complexity of gene dose regulatory mechanisms across vertebrates.



Breeding system influences mating-type proteins evolution in *Microbotryum* fungi

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POSTER SESSION 2 - P02.032, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

In numerous eukaryotic lineages, proteins involved in sexual compatibility are known to evolve faster than other proteins. In fungi, sexual compatibility is controlled by mating-type loci (MAT). In most basidiomycetes, there are two such loci: the P/R locus, encoding pheromone precursors and pheromone receptors, and the HD locus, encoding two homeodomain transcription factors, HD1 and HD2. In heterothallic fungi, mating can only occur between sporidia bearing distinct alleles at both of these loci. In some species, the great number of alleles at both loci results in a huge number of mating types. In most basidiomycetes, HD and P/R loci are found on distinct chromosomes, and the P/R loci is biallelic, showing an ancient trans-specific polymorphism. The HD locus shows distinct patterns depending on the species studied, being sometimes biallelic, most of the times multiallelic, and showing no wide-scale trans-specific polymorphism.

Microbotryum species, a.k.a. anther-smut fungi, infect and castrate a wide range of Caryophyllaceae species, and they are highly

selfing. The *Microbotryum* genus displays repeated independent events of linkage between HD and P/R loci. This results in two distinct breeding systems, either bifactorial, with unlinked HD and P/R loci, or unifactorial, with linked HD and P/R loci, which co-segregate. We studied the evolution of the HD genes, using the genomes of more than 30 *Microbotryum* species, with divergence times ranging from 0.4 to 11 million years. We show a strong influence of the breeding system on HD gene diversity and evolution: HD loci is multiallelic in bifactorial species, but biallelic in unifactorial species. We show that distinct parts of the HD genes show distinct evolutionary histories. Little trans-specific polymorphism is found outside of the HD1-HD2 interaction region, while this region, which acts as a single unit, shows an ancient trans-specific polymorphism. Most importantly, we show repeated independent events of HD loss of function in unifactorial species, with gene decays and even gene losses.



Disentangling the complex evolutionary history of sex chromosomes in snakes

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POSTER SESSION 2 - **P02.038**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

For a long time, snakes were presented as a textbook example of a group with gradual differentiation of homologous ZZ/ZW sex chromosomes. However, recent advances revealed that the ZZ/ZW sex chromosomes characterize only caenophidian snakes and certain species of boas and pythons have nonhomologous XX/XY sex chromosomes. We used genome coverage analysis in four non-caenophidian species to identify their sex chromosomes, and we examined the homology of sex chromosomes across phylogenetically informative snake lineages. We identified sex chromosomes, both XX/XY and ZZ/ZW, for the first time in 13 species of non-caenophidian snakes, providing much deeper insights into the evolutionary history of snake sex chromosomes. The evolution of sex chromosomes in snakes is more

complex than previously thought. In non-caenophidian snakes, it must have involved sex chromosome turnovers and changes in heterogamety, resulting in bigger overall variability of mostly poorly differentiated sex chromosome systems. In contrast, sex chromosomes of caenophidian snakes attained remarkable evolutionary stability once their differentiation proceeded. Moreover, snake lineages must differ in rates of sex chromosome differentiation as homologous sex chromosome systems of similar age but strikingly different differentiation levels were demonstrated. Lastly, certain genomic regions were likely co-opted as sex chromosomes in several snake lineages independently. All of these points make snakes an excellent vertebrate model group to study general aspects of sex chromosome evolution in the future.



DNA methylation patterns underlying paternal genome silencing in the mealybug *Planococcus citri*

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POSTER SESSION 2 - **P02.043**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Many organisms display sexually dimorphic traits regulated by differential expression and gene splicing. In the case of the mealybug *Planococcus citri*, sexual dimorphism is especially pronounced. Males and females differ dramatically in morphology, lifespan and the way they inherit genetic material. This is mediated by extreme sex-biased gene expression, despite mealybugs having no sex-specific genes, owing to a lack of sex chromosomes. This means that males and females are genetically identical, and therefore their differences are likely to be epigenetically regulated.

Mealybugs reproduce through paternal genome elimination (PGE), a form of genomic imprinting in which the haploid paternal genome in males is silenced in somatic cells and discarded from the germline, resulting in almost exclusively maternal expression and inheritance. The molecular mechanisms behind this paternal genome silencing are still under speculation, however previous research indicates DNA methylation may be involved. Mealybugs show distinct sex-specific DNA methylation profiles and considering the known involvement of DNA methylation in imprinting in mammals and plants, it is possible that it also plays a role in this species. Furthermore, mealybugs have independently evolved promoter methylation associated with gene silencing, a pattern more similar to mammals than to other insects. To further understand the role of DNA methylation in paternal genome silencing, we have combined RNA-seq and DNA methylation data along with parental single nucleotide polymorphism information to ascertain whether differential methylation occurs on the maternal or paternal chromosomes, and how this is linked to sex-biased gene expression as well as the differential behaviour of paternal chromosomes in males and females. Together these results will give insights into epigenetic regulation of sexual dimorphism and genomic imprinting in an insect with unique biology.



Elucidating molecular mechanisms of sex determination in stick insects

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POSTER SESSION 2 - **P02.045**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Sex determination is a key process in animal development, triggering the differentiation of primary and secondary sexual traits essential for reproduction. In animals, sex determination is controlled by highly conserved genes from the doublesex and mab-3 related transcription factor (DMRT) family. The gene doublesex was first discovered in the fly *Drosophila melanogaster*, followed by mab-3 in the worm *Caenorhabditis elegans* and finally the gene DMRT1 in vertebrates. DMRT genes underwent various duplication events and independently evolved sex determination functions in multiple animal taxa. In insects, sex determination is controlled by a cascade of genes that evolved around two genes called transformer and the DMRT family gene doublesex. Detailed molecular studies of these two genes in holometabolous insects suggested that insect sex determination is generally controlled via sex specific splicing. However, recent studies of a wider range of

insect species challenge this assumption. Here we investigated the evolution and sex-specific splicing and expression patterns of the genes doublesex and transformer in stick insects, a hemimetabolous insect order. Surprisingly, we found a duplication of the gene doublesex with both copies featuring similar expression patterns between the sexes and across tissues, and no evidence for sex specific splicing. A RNAi knockdown experiment targeting one of the two copies did not reveal any effect on male or female secondary sexual traits. By contrast, we found clear evidence for sex-specific splicing of the gene transformer. In combination with new findings in other hemimetabolous insect orders, our data show that sex determination mechanisms in these orders differ strikingly from those in holometabolous insects. Thereupon, the study of non-model insect species will be key for reconstructing the evolution of insect sex determination mechanisms.



Escape from a conserved system: Sex chromosome turnover in the Hispaniolan giant galliwasp (*Caribicus warreni*, Diploglossidae)

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POSTER SESSION 2 - **P02.054**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Anguimorphan reptiles possess one of the oldest known sex chromosome systems among amniotes, likely originating in their common ancestor during the Early Cretaceous. These ZZ/ZW sex chromosomes are conserved across monitor lizards (Varanidae), beaded lizards (Helodermatidae), and the alligator lizard *Abronia lythrochila* (Anguidae). However, recent studies suggest that the anguimorphan family Shinisauridae and a part of the family Anguidae do not share this ancestral system, likely due to independent sex chromosome turnovers. To investigate the evolution of sex chromosomes within this group, we analyzed the gene content of cytogenetically identified ZZ/ZW microchromosomes in the Hispaniolan giant galliwasp (*Caribicus warreni*,

Diploglossidae) using comparative genome coverage analyses. Unexpectedly, the sex chromosomes of *C. warreni* are not syntenic with the ancestral anguimorphan system. Further genome coverage comparisons with species from the genera *Varanus* and *Heloderma*, along with qPCR-based gene dose assays of Z-linked genes, confirmed the absence of homology between the ancestral and derived diploglossid sex chromosomes. These findings indicate that, despite long-term stability in some anguimorphan lineages, sex chromosome turnover has occurred in others, making anguimorph reptiles a rare example of both deep conservation and occasional replacement of sex chromosome systems over tens of millions of years.



Evolution of Translation GTPases: From LUCA to Extant Diversity

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POSTER SESSION 2 - **P02.030**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Translation is a fundamental biological process that decodes genetic information encoded in mRNA to synthesize proteins. Having emerged around 4 billion years ago, translation is one of the most conserved cellular components. In this study, we investigate the evolutionary history and diversity of bacterial translation factors - particularly translation GTPases - using comparative analyses with their archaeal and eukaryotic homologs. Showing striking homology among translation GTPases, we suggest a common ancestral origin, likely predating LUCA and show that the elongation and initiation functions can be traced back to an ancient generalist form of translation GTPases. Our comparative

sequence and structure analysis reveal that the universal translation GTPases possess extension segments, particularly at their N- and C-terminal regions. Although the functional roles of these extensions remain unclear, our findings suggest that translation GTPases have undergone lineage-specific adaptations in response to diverse environmental pressures, contributing to the divergence of translation systems among the three domains of life. By identifying novel molecular features across time, our work provides new insights into the evolutionary mechanisms that have shaped the translation machinery from early life to the present.



Faster-X evolution in globular springtails

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POSTER SESSION 2 - **P02.034**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The faster-X effect predicts that X chromosomes may evolve more rapidly than autosomes due to selection on expressed recessive alleles. Quantifying the effect empirically usually requires numerous assumptions due to the 'typical' biology of expression and inheritance, and so its strength remains unclear. Springtails present a novel opportunity to ameliorate this. Male globular springtails have hemizygous X chromosomes, diploid autosomes, and eliminate their paternal genome during spermatogenesis. This results in all chromosomes alternating between sexes and showing transmission bias between generations. Hence, the X chromosomes and autosomes only differ in

the fixation probability of male-beneficial alleles. We generated whole-genome resequencing and RNAseq data for the globular springtail *Allacma fusca*, to quantify the faster-X effect. We find that non-synonymous X-linked diversity is depleted relative to the autosomes. We infer the distribution of deleterious fitness effects (DFE) and find that new mutations on the X chromosomes show stronger fitness effects, indicative of greater purifying selection. Finally, accounting for the DFE, we see higher rates of adaptive evolution on the X chromosomes, supporting the faster-X effect.



From Genome to Function: Evidence of Transcriptional activity in Sciaridae Germline-Restricted Chromosomes

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POSTER SESSION 2 - **P02.044**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Various organisms eliminate large portions of their genome from somatic cells during early development; in extreme cases, entire chromosomes become restricted to the germline. These germline restricted chromosomes (GRCs) have evolved independently across the tree of life in diverse taxa, including hagfish, lamprey, passerine birds and three dipteran families: Cecidomyiidae (gall midges), Chironomidae (non-biting midges), and Sciaridae (fungus gnats). Recently, foundational genomic work has begun to unveil some of the mysteries behind GRC evolution in Sciaridae. Analysis in *Bradysia coprophila* has revealed that its two distinct GRCs are extremely gene-rich (~15,000 genes). Strikingly, whilst some of these genes are paralogs derived from somatic Sciarid core-chromosomes, many others appear phylogenetically distinct, with analysis placing them within the Cecidomyiid clade. Hence, Sciarid GRCs are presumed to have evolved from a hybridisation event between these two taxa. Whilst functional genomics in songbirds and lampreys has shown GRCs do express genes, often involved in germline cell development, differentiation and sex determination, no such work has been done on the Sciarid GRCs. Therefore, it remains an open and crucial question as to whether these large, and extremely gene rich chromosomes actively transcribe mRNA and produce functional proteins.

Here we address this question by combining genomic, transcriptomic and cytogenetic approaches to investigate whether transcription occurs from GRCs throughout various stages of *B. coprophila* development. We employ bulk RNA-seq on *B. coprophila* germline libraries (testes and

ovaries) in order to identify RNA reads mapping to GRC-linked scaffolds. Somatic libraries, which lack GRC RNA, served as controls to flag misaligned reads from highly similar core-chromosome paralogues. We stress that detecting GRC-gene expression using bulk RNA-seq is challenging. Reproductive tissue dissected from an individual contains a mix of somatic cells (containing only core-chromosomes) and germline cells (containing both GRCs and core chromosomes). Consequently, GRC transcripts are considerably underrepresented compared to those from core-chromosome. As a result, our approach likely detects only the most highly expressed GRC-linked genes.

We identify ~85 GRC-linked genes expressed throughout *B. coprophila* development, although this number falls to ~20 confidently expressed GRC-linked genes when accounting for core-chromosome read contamination. Of these confident GRC-linked genes, approximately half are expressed at the larval stages of development. We investigate the function of GRC genes using bioinformatic analyses and complement these findings with cytogenetic approaches to confirm gene expression and visualise the transcriptional activity of the GRC across developmental stages. The detection of only ~20 expressed genes out of over 15,000 annotated GRC-linked genes raises important questions about whether GRC transcription is truly limited to a small subset of genes, or whether more sensitive approaches—potentially targeting additional life stages or single-cell resolution—are needed to fully reveal their functional landscape.



Hermaphroditism in Insects; The Unbelievable Case of *Icerya purchasi*

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POSTER SESSION 2 - **P02.029**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Insects are one of the most diverse and widespread animal clades, harbouring almost all known mechanisms of sex determination. However, in stark contrast to its prevalence among many invertebrate groups, hermaphroditism in insects is virtually non-existent... except for a remarkable instance! The case of *Icerya purchasi*, a hemipteran plant parasite, commonly known as the cotton cushion scale insect.

I. purchasi is the only insect unequivocally described as androdioecious (i.e., its populations consist of male and hermaphrodite individuals) while also being a haplodiploid (i.e., males originate from unfertilized eggs). In this species, males are rare, making up just 0.01% to 10% of the population. The rest of the population is made up of hermaphrodite individuals, which despite looking like females, can produce both eggs and sperm at the same time. With self-fertilization as the dominant mode of reproduction, one can't help but wonder:

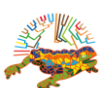
How can an otherwise fully female-looking and behaving individual produce sperm?

An enigma for the last 100 years, two main hypotheses have been competing to explain this biological puzzle. The prevailing

hypothesis posits that sperm in hermaphrodites originate from an established phenomenon called "polyspermy", in which multiple sperm enter an egg but only one fertilises it. The other sperm pronuclei persist and eventually form the sperm producing tissue in the adult hermaphrodite. Alternatively, the other hypothesis suggests that genome elimination in specific germline cells, during the hermaphrodite's development, produces the haploid sperm-producing tissue.

To unravel this mystery, we combined cytogenetic techniques with whole-genome computational analysis. Specifically, we used genomic staining methods such as DAPI and FISH to distinguish between the two proposed cytogenetic mechanisms. Then, to assess the paternal genetic contribution to the sperm tissue, we analyse genetic data from mother-offspring pairs.

These findings will elucidate the reproduction mechanisms of the only known androdioecious insect. Thus, paving the path for future population genetic inquiries into wild populations and allowing us to better understand the evolution of androdioecy, specifically in the context of insect haplodiploidy.



Insights into inheritance and genomic variation patterns following hybridisation between two divergent cichlid lineages

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POSTER SESSION 2 - P02.047, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Hybridisation plays a major role in speciation and formation of adaptive radiations by reshuffling genetic material. There can be a wide range of evolutionary consequences of such hybridisation events. It can give rise to species with novel adaptive traits and improved fitness compared to the parental lineages or it might also lead to accumulation of deleterious mutations that reduce the fitness of the hybrid species. Hybrid swarms are known to have played a key role in the rapid diversification of the East African cichlid radiations. Yet, how genomes from distantly related lineages interact to produce such variation remains only partially understood.

In this study we investigate a novel hybrid species that was produced by crossing two eco morphologically similar species but from distantly related lineages: *Maylandia emmiltos* from Lake Malawi and *Neochromis omnicaeruleus* from Lake Victoria. Both of these parental species are rock dwelling algae scrapers. Using long-read sequencing, we generated phased genome assemblies of hybrid individuals to reconstruct haploid parental genomes in the absence of reference genomes. Additionally we have also used short read sequencing for a large

number of the offsprings to study the effect of parental contributions in them.

Our analysis shows the variations segregating across the chromosomes in the hybrids is not always completely heterozygous. But in certain regions of the genome we observe an over representation of one parental lineage. This suggests asymmetric genomic contributions and potential selection acting on specific loci. Furthermore, we explored the occurrence of transposable elements (TEs) and their propagation across generations. Preliminary results indicate that there is a substantial amount of putative de novo transpositions that accumulate and require to be examined in more detail.

These findings highlight how hybridisation between divergent lineages not only combines distinct genetic backgrounds but also affects genome-level dynamics such as biased parental genome retention and TE proliferation. Our study aims to contribute towards a deeper understanding of how interactions between divergent genomes shape evolutionary outcomes in hybrid species.



IT'S A MATCH: using modelling to understand the evolution of fungal mating and incompatibility systems

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POSTER SESSION 2 - **P02.051**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Compatibility between fungal hyphae is governed by two contrasting systems depending on the life stage: During mating fungal hyphae seeks to fuse with genetically different hyphae and avoid selfing. On the other end, there are hyphae in the vegetative stage who only will allow fusion with itself and should reject non-self. Mating in fungi is governed by MAT-genes, increasing either the number MAT-loci or alleles at a given MAT-locus will increase the number of mating types which promotes outcrossing and increases the chance of mate compatibility. In the mushroom forming fungi a massive diversification in the number of alleles per MAT locus has taken place, but the number of MAT loci has not increased

beyond two. For vegetative compatibility, the opposite is the case. Here we find an increased number of incompatibility loci, but there are few alleles per loci. The current study attempts to use modelling and simulations to understand why we see a diversification in alleles but not in the number of loci for mating, and why the situation is reversed for vegetative compatibility. In the models, an increase in the number of alleles seemed to increase compatibility while promoting outcrossing, while an increase in the number of loci decreased compatibility and promoted outcrossing. In addition, we explore the effects that population size and population structure could have on these systems.



Massive genome expansion and rearrangement in an androdioecious scale insect

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POSTER SESSION 2 - P02.042, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The androdioecious mating system of the cottony cushion scale (*Icerya purchasi*) and other Iceryini are unique among insects, with populations consisting largely of selfing hermaphrodites, with rare haploid males allowing for occasional outcrossing. Selfing dramatically lowers effective population size, increasing both genetic drift and homozygosity across the genome, allowing novel variants to rapidly become homozygous. Due to the increased effect of drift, switches to selfing also initially allow transposable elements (TEs) to proliferate, in turn increasing genome size. However, on longer time scales genome size and TE load is observed to decrease in selfing and androdioecious species.

Compared to dioecious scale insects, the *Icerya purchasi* genome is 2 – 4 times larger, while its diploid chromosome count is smaller at 4, compared to the 6 – 34 chromosomes present in dioecious scale insects. To examine if androdioecy could have played a role in the expansion in *Icerya purchasi* genome size or affected genome structure we performed a comparative analysis of all scale insects sequenced to a chromosomal level. We found TE proliferation was responsible for much of

the increase in *Icerya* genome size. However, we observed a similar increase in TE load and genome size in an outcrossing species from the same family (Monophlebidae), suggesting this increase in genome size may have begun before the switch in mating system.

Within most families we found the chromosomal synteny of scale insects to be reasonably well conserved. In contrast, we found an abundance of large intrachromosomal rearrangements in *Icerya purchasi* since its divergence from dioecious species. Previous studies have found increased genome reshuffling in other Hemiptera with a reproductive mode which dramatically decreases outcrossing (parthenogenesis). This increase in chromosomal shuffling observed in *Icerya* may in part be due to the increased load of highly similar TEs, well known catalysts of chromosomal rearrangements through ectopic recombination. Additionally, selfing allows for massive chromosomal rearrangements to become homozygous within two generations, thereby reducing any negative fitness consequences of being heterozygous. Our results suggest androdioecy may have played a role in the massive genomic reshuffling present in the *Icerya* genome.



Mechanisms of transition to parthenogenesis in hybrid stick insects

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POSTER SESSION 2 - **P02.039**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Sexual reproduction is the most widespread mode of reproduction in multicellular organisms. But despite the prevalence of sex, many lineages have successfully evolved alternative reproductive strategies. Yet, the underlying mechanisms associated with transitions to alternative reproduction remain poorly understood, despite their various genomic and evolutionary consequences. For instance, certain non-sexual reproductive modes preserve heterozygosity, whereas others do not. As recessive load is expected to be associated with fertility costs, this can impact the success and persistence of non-sexual lineages. The elucidation of the cellular mechanisms that govern non-sexual reproduction consequently enhances our comprehension of their evolution. *Bacillus* stick insects are a unique genus characterized by a diverse array of reproductive strategies, including parthenogenesis, where females generate fertile eggs in the absence of fertilization, and hybridogenesis, where the maternal genome is clonally transmitted to fertilized eggs. Our

research team reconstructed the phylogenetic history of the genus, showing multiple transitions between various reproductive modes and ploidy levels. To understand the cellular mechanisms underlying these transitions, we characterized a *Bacillus* lineage that reflects an intermediate state in these transitions. This lineage is diploid, parthenogenetic, and a hybrid. By cytological reconstruction of the progression of meiosis, we demonstrated that the genome is duplicated before meiosis ("premeiotic replication"). This enables copies of identical chromosomes to pair instead of homologs from a different parental species. In those hybrids, recombination can therefore occur without loss of heterozygosity. Additionally, this mechanism enables transitions to triploidy and the generation of new parthenogenetic lineages. These results shed light on how transitions to asexual reproduction can occur in insects and more broadly strengthen the evidence that premeiotic replication is a common mechanism of parthenogenesis in hybrids.



Mitochondrial RNA interference in the blue mussel: barriers to overcome

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POSTER SESSION 2 - **P02.027**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The blue mussel (*Mytilus edulis*) is an ideal model for studying the evolution of mitochondrial genomes (mtDNA) and the emergence of novel mitochondrial genes in animals. While animal mtDNA is typically conserved in terms of gene content, notable exceptions exist in bivalves exhibiting doubly uniparental inheritance (DUI) of their mitochondria, such as *M. edulis*. In this species, two novel protein-coding genes have evolved: M-ORF, found in the male-transmitted mtDNA, and F-ORF, present in the female-transmitted mtDNA. Although the functions of these genes remain unknown, they are suspected to be involved in sex determination.

To investigate their roles, RNA interference (RNAi) presents a promising approach by enabling gene silencing in mussel cells. The goal of our research is to develop an RNAi-based method to target mitochondrial genes, such as M-ORF and F-ORF, in blue mussel primary cell cultures. This project poses

significant challenges: *M. edulis* is not a standard model organism in molecular biology, no immortalized cell lines are available, and RNAi must take place within mitochondria, something achieved in only a handful of studies to date.

In this presentation, I will highlight key experimental achievements, including the successful inhibition of the nuclear gene β -actin in *M. edulis* primary cell cultures, and preliminary evidence suggesting the silencing of F-ORF, a mitochondrial gene of unknown function. I will also address current obstacles and propose strategies to overcome them, such as improving the consistency and reproducibility of mitochondrial RNAi. Establishing an effective mitochondrial RNAi protocol in mussels will allow for the study of sex-specific genes like F-ORF and M-ORF, and their potential roles in sexual differentiation in DUI bivalves.



Mitonuclear coevolution as a driver of latitudinal avian speciation trends

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POSTER SESSION 2 - **P02.028**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Reciprocal selection between the nuclear and mitochondrial genomes—mitonuclear coevolution—maintains compatibility between nuclear- and mitochondrially-encoded products that associate within the mitochondria during organelle functions. This process is predicted to be an important driver of eukaryotic speciation due to its production of distinct genetic haplotypes that form the basis for severe genetic incompatibilities (“mitonuclear incompatibilities”) and strong reproductive barriers between taxa. However, though mitonuclear incompatibilities have been detected in various systems, the proposed connection between mitonuclear coevolution and speciation remains tenuous. In this study, we investigated the association between these processes by examining mitonuclear coevolutionary dynamics in the context of a major, latitudinal avian speciation trend. Previous research has found that speciation occurs more rapidly in temperate, high-latitude avian systems compared to tropical, low-latitude avian systems across the Americas. These high-latitude avians experience more variable temperature conditions and often participate in long-distance migration which both place additional stress on mitochondrial function. As such, temperate avian systems may experience stronger selection for efficient mitonuclear coevolution compared to their tropical counterparts which could lead to faster speciation rates. To evaluate this hypothesis, we examined patterns of mitonuclear

coevolution across high- and low-latitude avian systems using a comparative genomic approach that incorporated whole-genome sequence data from thirty-nine pairs of closely related avian taxa. We utilized Bayesian codon evolution models and pairwise dN/dS ratio comparisons to assess genomic signals of mitonuclear coevolution across the avian phylogeny and in each species pair respectively. We then compared the genetic patterns associated with temperate and tropical avian pairs to determine whether selection for mitonuclear coevolution varies across latitudes. In our preliminary analyses, we identified strong support for mitonuclear coevolution having occurred across the avian phylogeny and within each avian species pair consistent with theory claiming that mitonuclear coevolution is ubiquitous across eukaryotic organisms. However, we did not find evidence that selection for mitonuclear coevolution differs between temperate and tropical avian systems. This finding implies that mitonuclear coevolution may not be driving the latitudinal gradient in avian speciation though this process may still reinforce the ecological and evolutionary processes that are responsible for this trend. Our research provides valuable insights on mitonuclear coevolutionary dynamics in avian systems, which are applicable across Eukarya, and greater perspective on the potential influence of mitonuclear coevolution in wider evolutionary processes such as speciation.



Mutational Biases and Selection in Mitochondrial Genomes: Insights from a Comparative Analysis of Natural and Experimental Populations of *Caenorhabditis elegans*

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POSTER SESSION 2 - **P02.037**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Random mutations display biases in their relative frequencies with important consequences for genome structure and composition. While laboratory studies have provided insights into the spectrum of spontaneous mutations, the laboratory environment of optimal growth conditions may produce its own biases. Hence, further investigation is necessary to determine if laboratory results are truly representative of natural populations. We analyzed the mitochondrial (mtDNA) genomes of 1,524 *Caenorhabditis elegans* natural isolates comprising 550 unique mtDNA haplotypes to investigate patterns of mtDNA evolution in the wild. Ancestral reconstruction was used to polarize 2641 mutations (90 indels, 2,551 SNPs) and the results were compared to mutations identified in experimentally evolved laboratory populations under minimized selection. The frequency of mutations is associated with both codon position and site degeneracy in a manner consistent with strong purifying selection on mtDNA protein-coding sequences. There is significant variation in the per site nonsynonymous and synonymous

substitutions between genes and more particularly genes from ETC complex I are particularly enriched in nonsynonymous substitutions, namely nd-6 and nd-2. The probability of mutation is higher at sites with flanking G/C and the per gene synonymous mutation frequency is associated with GC content at 1st and 2nd base positions in codons, but not with GC content at 3rd base position. Furthermore, the position within a gene influences the base substitution frequency as the 5' and 3' ends of genes have lower synonymous substitution frequency than their centers. We also report the first cases of large heteroplasmic mtDNA structural variants, possibly occurring in natural populations of *C. elegans*, including a suspected duplication of the origin of replication. Although some of the patterns of mtDNA mutational bias are similar between the laboratory and wild populations, we observe significant differences. In particular, transversions typically associated with oxidative damage are less common at four-fold degenerate sites in natural populations than in the laboratory.



Recurrent centromere repositioning in an otherwise stable karyotype of birds

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POSTER SESSION 2 - **P02.050**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Centromeres are structural features of chromosomes that act as the primary binding site for the kinetochore during cell division. In birds, like in many other animals, the centromeric DNA is mainly composed of satellite arrays that can expand across megabases. Previous studies have observed that centromeres can shift epigenetically to a different location on the chromosome that may over time accumulate repeats and become established as evolutionary new centromeres (ENCs). ENCs can play important roles in evolutionary processes, such as speciation, but our understanding of the mechanisms driving ENC evolution and how they get established within karyotypes is limited. Because of difficulties in assembling such complex centromeres, reconciling molecular and cytogenetic methods with genomic data is often problematic. Here we investigate a highly syntenic microchromosome of songbirds and its

evolutionary history. We find that two independent centromere shifts have occurred on chromosome 18: one in the ancestor of the families Corvidae and Laniidae, and the second in the sister species, the collared flycatcher (*Ficedula albicollis*) and the pied flycatcher (*F. hypoleuca*). Both shifts resulted in megabase-scale valleys of recombination rate in regions with otherwise high recombination rates. These findings on an otherwise highly collinear chromosome suggest that the ENCs have shifted without other rearrangements. Our results show that our bioinformatics approaches provide a useful tool to study centromeres and ENCs, even in cases where they are largely missing from assemblies. The methods presented in this study will also aid future research of centromere and ENC identification in non-model organisms.



Repeated evolution of all-female populations in facultatively parthenogenetic stick insects

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POSTER SESSION 2 - **P02.041**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Sexual reproduction is the most common reproductive mode in eukaryotes. However, despite well-supported long-term benefits of sexual reproduction, its short-term benefits – preventing the invasion by asexual mutants – remain poorly understood. One possible explanation for the short-term persistence of sex is that transitions to parthenogenesis are difficult because they require the crossing of fitness valleys. In addition, asexual reproduction may entail short-term fitness costs, but such costs have rarely been quantified in natural populations. Here, we leverage the multiple local transitions to all-female populations (enforcing asexuality) in the facultatively parthenogenetic stick insect *Bacillus rossius* to quantify potential short-term costs associated with transitions between reproductive modes and with asexual reproduction. Females in sexual

populations of this species are able to reproduce via parthenogenesis, but only do so if they remain virgin. Specifically, we sampled individuals from seven pairs of sexual and asexual populations. To quantify potential costs of asexual reproduction, we compared fertility and fecundity between (virgin) asexual and mated sexual females in a common garden environment to identify differences in fitness distributions. To quantify the costs associated with transitions between sexual and asexual reproduction, we further contrasted fitness distributions of virgin sexual females with those of mated asexual ones. Finally, using multiple population pairs within a single species allowed us to disentangle reproductive-mode effects from population-specific ones, and to replicate analyses in an unprecedented manner.



Sexual conflict and the evolution of unconventional sex-roles and male pregnancy in pipefish

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POSTER SESSION 2 - **P02.046**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Pipefish, alongside seahorses and sea dragons, belong to the teleost family Syngnathidae where males carry broods through specialized skin attachments or brood pouches in varying levels of complexity, a unique form of parental investment akin to pregnancy. Additionally, some members have evolved reversed sex-roles where females compete for access to mates. The evolution of such sex roles is coupled with the evolution of sex-specific traits, which arise from differing selection on males and females. These traits although sex-specific, evolve from one genome shared by both sexes, thus contend with diverging fitness optima between males and females. This creates sexually antagonistic conflict (SAC) which can be alleviated or resolved through sex-biased expression of genes. When sex is genetically determined,

sex specific inheritance of sex chromosomes allow for sex-bias to evolve more freely and accumulate. Furthermore, SAC can achieve full resolution on sex chromosomes when alleles are limited to the sex they benefit. Sex-role reversed pipefish provide the unique opportunity to study sexual selection in females and pregnancy in males. In this study, we looked for signals of sex chromosomes by comparing male and female pool-seq data of two pipefish with sex-role reversal, the straightnose pipefish (*N. ophidion*) and broadnose pipefish (*S. typhle*). We further examined the transcription profiles of four tissues in females and in pregnant and non-pregnant males to understand the contribution of sex-biased expression to sex-specific traits.



Sharks and rays have the oldest vertebrate sex chromosome with unique sex determination mechanisms

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POSTER SESSION 2 - **P02.033**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Sex determination has been investigated across vertebrate lineages to reveal stepwise evolution of sex chromosomes and diversity of responsible molecular mechanisms. However, these studies hardly encompass cartilaginous fishes deeply isolated from the rest of vertebrates, which hinders the comprehensive view of vertebrate sex determination. Here, we produced chromosome-scale genome assemblies of egg-laying shark species and comparatively investigated genome sequences and transcriptome profiles across diverse cartilaginous fishes. Sex chromosome identification, supported by cytogenetic experiments, elucidated the homology of X chromosomes between sharks and rays as well as an extensively degenerating Y chromosome harboring no male-specific genes. These sex chromosomes scarcely

included orthologs of previously documented sex determining genes. Transcriptomic analyses combined with histology of embryonic gonads revealed female-biased expression of X-linked genes including those implicated in TGF- β and IGF signaling pathways, which are attributed to their incomplete dosage compensation. Our findings indicate that sharks and rays share the oldest sex chromosomes among vertebrates that originated around 300 million years ago and the dosage-dependent sex determination mechanism comprised of distinct molecules from other vertebrates. This study highlights the antiquity of sex chromosomes and uniqueness of sex determination mechanisms in sharks and rays, which advance our understanding of the evolutionary plasticity of vertebrate sex determination.



The evolution of reproductive mode in the *Carassius auratus* complex

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POSTER SESSION 2 - **P02.026**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Abstract: Freshwater fish of the genus *Carassius* are widely distributed across Asia, Europe, and Australia. This includes the *Carassius auratus* complex, a phylogenetically diverse species group with uncertain taxonomy. The *C. auratus* complex is native to Asia but has been introduced to non-native habitats worldwide. It comprises both sexually reproducing diploid individuals and polyploid individuals that predominantly reproduce asexually through gynogenesis. The evolutionary relationships between these different reproductive modes, as well as the invasion history of the various lineages, are poorly understood. A phylogenetic approach using mitochondrial DNA (Cyt b sequence) in combination with six microsatellite markers was employed to investigate the invasion history of different lineages and reproductive modes and to explore the evolutionary relationships between them. The Cyt b analysis was based on sequences from 10 native populations in China and 35 invasive populations in Europe and Australia. Additionally, 1,427 individuals representing 93 populations from China, Europe, and Australia were genotyped at six microsatellite loci to determine their ploidy level and reproductive mode. Phylogenies of three nuclear genes were also used to test two hypotheses regarding the origin of triploid lineages: allopolyploidy and autopolyploidy.

Our results suggest that the invasion of the *C. auratus* complex in Europe occurred through two routes, possibly driven by both historical and anthropogenic events. The microsatellite analysis revealed that 67.19% of individuals were triploids and 33.8% were diploids among the 1,427 individuals

studied. Interestingly, most native populations consisted of a mix of diploids and triploids, with triploids predominating. In contrast, invasive populations exhibited considerable variation in reproductive modes, ranging from purely diploid to purely triploid and mixed populations. Additionally, diploids were dominant in older Eastern European populations, while triploids were dominant in more recently established Central European populations. Both mtDNA and microsatellite markers revealed no separation at the ploidy level, as alleles were shared by both diploid and triploid individuals, and sympatric diploids and triploids shared mtDNA haplotypes in seven highly diverged lineages and sublineages. Furthermore, the high genetic diversity observed in triploid forms may result from propagule vectors, which help mitigate the genetic bottleneck effects typically seen in successfully invasive species. The origin of gynogens could not be conclusively determined using nuclear DNA (Rag1 and S7 genes), as Rag1 analysis suggested an allopolyploid origin, while S7 analysis pointed to an autopolyploid origin.

The *C. auratus* complex provides an ideal model for studying the reproductive traits of polyploid triploids, which holds significant value in animal genetics and reproductive biology.

Key Words: *C. auratus* complex, *C. a. auratus*, *C. a. gibelio*, reproductive mode, gynogenesis, invasive, gene duplication, whole genome duplication, polyploidy, PCR, microsatellites, mtDNA, Cyt b, allele, locus, loci, diploid, triploid, allopolyploid, autopolyploid, nDNA.



The genomic predictability of hybridization: insights from pine contact zones across contrasting environments

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POSTER SESSION 2 - **P02.048**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Hybridization is increasingly recognized as a key driver of evolutionary process that can facilitate adaptive introgression shape the genomic architecture, influence species boundaries, and promote the emergence of novel lineages. In long-lived forest trees with weak reproductive barriers, such as pines, hybrid zones provide natural laboratories for understanding these dynamics. However, the extent to which hybridization outcomes are ecologically repeatable and evolutionarily deterministic remains poorly resolved. Here, we investigate hybrid zone dynamics between Scots pine (*Pinus sylvestris*) and members of the *Pinus mugo* complex across contrasting habitats in Central Europe —taxa characterized by overlapping distributions and frequent natural hybridization. We ask how repeatable is hybrid genomic composition across environments, how selection acts on admixed genomes and whether convergent molecular patterns are evolving in those zones.

Materials and Methods: We sampled over 1700 individuals across 29 populations, including three replicated hybrid zones in Central Europe - two located in peat bogs and one on a sandstone outcrop, capturing variation in ecological context, alongside allopatric reference populations of *Pinus sylvestris*, *P. mugo*, and *P. uliginosa*. Individuals were genotyped at over 7,000 SNP loci using a custom array targeting transcribed and functionally annotated genomic regions. We assessed genetic structure, levels of admixture, and hybrid classification using multivariate and ancestry-based approaches. Hybrid classes were further characterized by estimating genomic ancestry proportions and examining clinal variation across loci. To detect potential targets of selection, we applied complementary genome scan methods and identified outlier loci associated with ancestry and environmental context. These analyses were integrated with chloroplast DNA data to infer the directionality of gene flow. Functional annotations of candidate loci were used to explore the genomic basis of local adaptation and introgression dynamics.

Results: All three hybrid zones exhibited extensive and ongoing hybridization, dominated by advanced-generation hybrids and backcrosses, with few F1 individuals. Genomic structure was strikingly consistent across sites, with asymmetric introgression favoring *P. mugo* ancestry, even in ecologically distinct contexts. Individuals morphologically resembling *P. uliginosa* were genomically indistinguishable from late-generation hybrids, lacking unique ancestry components. Despite strong parallelism in hybrid genomic composition, site-specific patterns of genetic diversity and introgression were evident. Overall, pure *P. sylvestris* and *P. sylvestris*-like individuals exhibited elevated genetic differentiation and higher proportions of loci under selection. Genomic clines revealed non-neutral introgression at several outlier loci, suggesting environment-dependent selection favoring *P. mugo*-derived alleles in certain genomic regions. Patterns of chloroplast inheritance and hybrid index distributions supported recurrent, asymmetric gene flow from *P. mugo* into *P. sylvestris* genomes.

Conclusions: This study provides compelling genomic evidence for the repeatability of hybridization outcomes across replicated hybrid zones in a forest tree system. While hybrid genomic composition was strikingly consistent across sites, local selection imposed differential pressures on admixed genomes—particularly those with predominant *Pinus sylvestris* ancestry in peatland habitats. These findings highlight the interaction between hybridization and ecological selection in shaping hybrid lineage trajectories and influencing the stability of hybrid zones. Our results underscore the importance of ecological context in driving non-neutral introgression and contribute to broader discussions on species boundaries and conservation within the *Pinus mugo* complex. More broadly, this study illustrates the power of replicated hybrid zones as natural experiments for unraveling the genomic architecture of hybridization and its evolutionary consequences in the speciation continu



Unexpected diversity of telomeric repeats in Andrena bees

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POSTER SESSION 2 - P02.052, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The telomerase based system is a frequently found solution to the end-replication problem of linear chromosomes. The corresponding complex consisting of a non-coding telomerase RNA (TR) and a telomerase reverse transcriptase (TERT) is able to add characteristic telomeric tandem repeats to chromosome ends. Assuming the existence of one TR gene per species, recent studies reported a large diversity in the sequence of telomeric repeats in some insects including a few species of the *Andrena* genus. These ground nesting, solitary bees are widespread and very important pollinators in natural as well as agricultural ecosystems.

I will present the results of our detailed computational analysis of *Andrena* bee genomes and the underlying DNA sequencing data. We found multiple copies

of TR genes forming distinct telomeric tandem repeats in the majority of currently sequenced *Andrena*. Although recently reported for plants, to our knowledge this is the first time this phenomenon has been observed in animals. Findings such as alternating patterns of different tandem repeat sequences at chromosome ends and the experimental evidence of co-expressed presumably functional, i.e. over-represented tandem repeats, and dysfunctional TR gene copies add another, previously unexpected, layer of complexity to the evolution of telomeric repeats.

Although *Andrena* represent one of the largest animal genera only a dozen sequenced genomes are currently available. We are looking for research groups working with *Andrena* bees to jointly study the evolution of telomerase RNA in this genus and beyond.



Age-dependent sex under mutation accumulation

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MEETING ROOM 118+119, August 21, 2025, 10:30 AM - 12:30 PM

An often-observed strategy among facultatively sexual organisms is to invest in (costly) sex only when in poor condition — a scenario usually caused by environmental change, population crowding, or other external cues. Yet, a major determinant of organismal condition is internal: mutation accumulation with age. Whereas somatic mutations can result in deteriorated organismal condition, germline mutations strongly impact the fate of a lineage, as they can be transmitted to the offspring to varying degrees, depending on the reproductive mode. As such, switching between reproductive modes based on germline state can help maximize the benefits of sexual reproduction while minimizing its costs. However, an organism cannot always accurately measure the state of its germline based on its somatic condition. In such cases, age might emerge as a reliable cue due to its correlation with mutation load. Such age-dependent plasticity in reproductive mode has previously been described empirically in *Hydra oligactis*. In this talk, I will describe a mathematical model constructed to understand the population and evolutionary dynamics of three reproductive strategies: sexual reproduction when young followed by

asexual reproduction when old (sex only when young), asexual reproduction when young followed by sexual reproduction when old (sex only when old), and obligate sex. We show that all strategies can all persist in isolation, under different parameter regimes. The two age-dependent facultative sex strategies are by far the dominant evolutionary outcomes due to an "exploitation" effect that operates when they interact with obligate sexuals, allowing them to enjoy the benefits of sex (via fusion with gametes produced by the exploited) without paying as much of a cost. This leads to three outcomes under different parameter combinations: (i) over-exploitation leading to the extinction of a strategy that is viable in isolation, (ii) priority effects where evolutionary outcome is determined by whether a strategy is the resident or not, and (iii) sex only when old being the dominant outcome. These results contribute to our understanding of the evolution of sex, demonstrating not only the viability of age as a cue for facultatively sexual organisms, but also its inherent differences compared to condition-based cues.



Characterizing the germline-restricted chromosome in a hybrid-species complex of sparrows

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New species can emerge from hybridization without a change in chromosome numbers, and such a new species is a type of hybrid species. The evolution of the hybrid genomes offers immense opportunities to study genetic (in)compatibility, adaptation, and speciation. A recently discovered feature of the over 5000 songbird species—the germline-restricted chromosome (GRC)—is present only in germline cells and absent from somatic cells, meaning it has been overlooked in most songbird genomic studies to date. Interestingly, the GRC typically follows maternal inheritance patterns, with occasional cases of paternal inheritance. Furthermore, its copy number in females vs. males are reminiscent of an XX/XO sex chromosome system. These characteristics make it particularly intriguing in the context of the emergence and evolution of selfish genetic elements, as well as potential genetic incompatibilities with other maternally inherited elements, such as the mitochondrial genome.

In this study, we investigate the GRC in a young hybrid songbird (~5,800 years old), the

Italian sparrow *Passer italiae*, and its two parental species, the house sparrow *P. domesticus* and the Spanish sparrow *P. hispaniolensis*. Using a combination of PacBio HiFi long reads, Nanopore ultra-long reads and TELL-seq linked reads, we characterized the gene content, diversity and mutation rate of the GRC across species to explore its evolutionary dynamics in the context of hybrid speciation. Our three sampled Italian sparrows show mitochondrial haplotypes similar to those of the parental house sparrow. As expected from the typical matrilineal inheritance, the Italian sparrow GRCs appear to have also arisen from house sparrow, however, followed by multiple episodes of complex structural mutations highlighting how much faster than the rest of the genome the GRC evolves. Our findings provide critical insights into the role of the GRC in genome evolution, and its potential relationship with the formation and stability of hybrid species. This work establishes a novel model system for studying the evolutionary impact of germline-specific chromosomes in birds.



Disentangling the genomic architecture of sex chromosomes in an androdioecious species with two mating types

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Androdioecy is a rare reproductive system in which both male individuals and hermaphrodites coexist within a species. The requirements for its emergence and long-term stability are thought to be strict, hence, it is very uncommon among living organisms. An exception is found in the Oleaceae plant family, which contains a large subset of all known androdioecious species. The evolutionary puzzle of androdioecy in this family was illuminated by the discovery of a highly conserved self-incompatibility (SI) system in which hermaphrodites are divided into just two cross-compatible groups. Male individuals can bypass this SI barrier, giving them a direct reproductive advantage that offsets the loss of female function. This shift alters the dynamics of sexual antagonism

between the reproductive morphs and may influence the evolutionary path of sex chromosomes. Recent efforts have revealed the hemizygous structure, mechanism, and conservation of this SI system across the family. To further investigate these dynamics, we mapped, assembled, and annotated the sex chromosome of the androdioecious *Phillyrea angustifolia*. Our analysis revealed a relatively small, yet extensively rearranged, sex-determination region. Ongoing work is focused on understanding how this region evolved from its autosomal ancestor and how it has developed epistatic interactions with the genomic region responsible for self-incompatibility.



Evolution of divergent male and female mitochondrial genomes in a basal protobranch bivalve *Yoldia hyperborea*

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Doubly uniparental inheritance (DUI) of mitochondrial DNA (mtDNA) is a mode of mtDNA transmission whereby the male mitogenome is inherited predominately by males and the female mitogenome is inherited by females. DUI is unique to bivalve mollusks and has been identified in all major bivalve clades, including Protobranchia, the most basal bivalve subclade. While this system has evolved in bivalves independently at least tens of times, the mechanics of this process is poorly understood. Here, we focus on the early stages of divergence of male and female mitochondrial genomes by sequencing them in a basal Protobranchia species *Yoldia hyperborea* where they are characterized by one of the lowest known level of divergence, suggesting a recent origin of this system. Despite the high relatedness of female and

male genomes, we find that they have undergone rapid divergence both on the sequence and the structural level, including an origin of a novel gene in the male genome. This novel gene is functional, as evidenced by the fact that it is transcribed and is subject to purifying selection. Additionally, the female mitogenome contained a duplicated cassette containing the cytochrome b gene, resulting in two different transcripts in female gonads both of which are subject to purifying selection, although their transcription levels differ by two orders of magnitude. These results showcase the rapid adaptation of female and male mitochondrial genomes soon after their separation in a double uniparental inheritance system, akin to that observed in evolution of sex chromosomes.



Evolutionary drivers and consequences of monogeny: a unusual reproductive system in fungus gnats

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Black-winged fungus gnats (Family: Sciaridae) are a group of Dipteran insects extraordinarily unique in their reproduction. They exhibit maternal genetic sex determination, where maternal genotype - instead of zygotic genotype - determines zygotic sex. Even more unusually, some species are monogenic, in which females produce offspring of only one sex. Other fungus gnat species are digenic and produce mixed-sex broods, but the intra-family sex ratio is often far from 50/50. These mating systems have not just evolved once, but experience extensive turnover within the clade, with both strategies being found in pairs of sister species.

Being a significant departure from the long-held dogma of stable, ancient and zygotic genetic sex determination systems, fungus gnats provide novel insights into general principles. For one, a central debate in this field is the evolution of sex-determining regions, for which fungus gnats provide a unique context for study. The genetic basis of maternal sex determination is a X-linked inversion heterozygous in female-producing females. This supergene seems to have

arisen independently in each instance of monogeny, and harbours intraspecific polymorphism. Does this non-recombining region accumulate deleterious mutations, or facilitate alleles advantageous for specialisation into female-production? While the evolutionary dynamics of this inversion is likely a key factor for the instability of monogeny, it also provides an intriguing parallel to the evolutionary drivers and outcomes of zygotic genetic sex determination regions.

To understand the impacts of specialising into single-sex production, and its interplay with mating system turnover, I focus on a model species *Bradysia coprophila*. I present findings on gene expression and phenotypic divergence between reproductive morphs, suggesting possible specialisation under selection. I also outline ongoing comparative studies of a closely-related species with independently-evolved monogeny and supergene polymorphism. This intriguing system broadens our understanding of the diversity of sex determination systems, and acts as an informative intra-sex analogy for sex chromosome evolution.



How parasites can influence recombination in an almost fully parthenogenetic population : the case of the Bedegar gall wasp.

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Cynipoidea are a group of hymenopterans that exhibit a wide diversity of reproductive strategies. Most species classically reproduce through haplodiploidy, typical of hymenopterans, where males are haploid, resulting from arrhenotokous parthenogenesis, and females are diploid, resulting from sexual reproduction. However, some species present different strategies, notably thelytoky, where males are typically absent and females can produce female offspring without reproduction. Even thelytoky can occur in various ways, either during meiosis (automixis) or without meiosis (apomixis). Two distantly related clades have even evolved cyclical parthenogenesis, where two generations are produced alternately each year: one thelytokous and one sexual. The rose bedeguar wasp, *Diplolepis rosae*, is a cynipid wasp that induces galls on wild roses. In many populations across Europe,

due to thelytoky, very few males are observed emerging from the galls. Consequently, the species shows a high level of homozygosity. However, we found that among two divergent populations, one retains some genome-wide heterozygosity. Selection scans could show that genes related to male function are under negative selection in the more diverse population and relaxed selection in the other population. This is potentially indicative of a selective pressure to maintain the ability to produce males. We indeed found that the more genetically diverse population also experienced much less parasitic pressure than the homozygous one. The persistence of highly homozygous lineages could be due to higher fertility, as shown by a tendency to produce larger galls. Our study shows how parasite pressure could potentially influence the maintenance of diverse genetic systems.



Stepwise recombination suppression around the mating-type locus associated with a diploid-like life cycle in *Schizothecium* fungi

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Recombination suppression often evolves around sex-determining loci and extends stepwise, resulting in adjacent regions with different levels of divergence between sex chromosomes, called evolutionary strata.

In Ascomycota fungi, evolutionary strata around the mating-type locus have only been found so far in few species, all with a rare diploid-like life cycle, known as pseudo-homothallic species, characterized by mycelia carrying nuclei of both mating types. In contrast, no recombination suppression has been observed in Ascomycota fungi with haploid-like life cycle and heterothallic genetic systems (with mycelia colonies of a single mating type each).

We investigated the evolution of recombination suppression in a clade of dung fungi encompassing 16 pseudo-homothallic and three heterothallic sibling species from the *Schizothecium* genus (Ascomycota, Sordariales).

The analysis of genetic divergence based on genome sequencing indicated recombination suppression around the mating-type locus in all studied pseudo-homothallic species. The non-

recombining region ranged from 600 kb to 1.6 Mb and harbored multiple evolutionary strata, varying in size and number among species. The separation of alleles associated with alternative mating types in gene genealogies, the high linkage disequilibrium and an inversion in one species altogether supported the lack of recombination in the mating-type locus proximal region in pseudo-homothallic species. The lack of trans-specific polymorphism suggested multiple independent events of recombination suppression or the occurrence of rare events of recombination or gene conversion. Progeny analyses showed the occurrence of recombination close to the mating-type locus in heterothallic strains.

We thus revealed multiple and likely independent evolutionary strata, associated with an extended diploid-like stage in *Schizothecium* fungi, which supports the hypothesis of an association between a pseudo-homothallic genetic system and recombination suppression around the mating-type locus and provides a good model for research on sex-related chromosome evolution.



The cheater-driven evolution of reproductive division of labour

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Background: Reproductive division of labour (RDL), where sterile 'helpers' assist specialised 'reproducers' in propagating genetic information, has repeatedly evolved across vastly different biological scales, from molecules to multicellular organisms. Traditionally, the evolution of RDL has been thought to require high relatedness between interacting individuals, as it entails altruism — helpers foregoing self-reproduction to benefit reproducers. Contesting this traditional view, we present a model predicting that RDL evolves when relatedness is sufficiently low.

Model: We constructed an individual-based model of evolving multicellular organisms. The model consists of a population of competing organisms. Each organism consists of a population of cells that divide and die. Each cell possesses a finite amount of resource to allocate between cell division and somatic function, with the allocation strategy being evolvable. Allocating to cell division increases the cell's relative fitness within the encompassing organism, whereas allocating to somatic function can increase the fitness of all cells in the same organism — constituting a trade-off between individual and collective benefit. Every cell exists in one of two interconvertible states with evolvable conversion rates. These cell states are associated with independent resource allocation strategies, which are initially identical but may diverge through evolution. Consequently, a cell may vary its resource allocation depending on its state.

Results: Simulations show that RDL evolves when relatedness is initially sufficiently low. The evolved RDL involves cell-state-dependent resource allocation, where cells in one state (helpers) allocate their resource to somatic function, whereas cells in the other state (reproducers) allocate it exclusively to cell division. Crucially, RDL causes an increase in relatedness among cells, thereby protecting organisms against the evolution of cheater

cells — cells that prioritise individual gain over collective benefit. This protection arises from two additional features involved in the evolved RDL. First, reproducer cells convert to helper cells, but not vice versa — i.e., conversion is irreversible. Second, reproducer cells are less abundant than helper cells. These features restrict the transmission of genetic information to a limited number of reproducer cells per organism, thereby reducing the within-organism effective population size from which cheaters may arise. We show that RDL evolves to provide protection against cheater evolution under both accelerating and decelerating returns on resource investment, demonstrating that this evolutionary outcome is robust to the shape of the trade-off curve.

Conclusions: Our model shows that RDL evolves as an evolutionary strategy to elevate relatedness and mitigate the threat of cheater evolution. This cheater-driven perspective predicts that RDL evolves when relatedness is low because the protective benefit of RDL rises with the risk of cheater evolution. Therefore, while our model agrees with kin selection theory in predicting that high relatedness coincides with RDL, it challenges the prevailing view by predicting that high relatedness is a consequence, rather than a cause, of RDL evolution.

Our model offers a general mechanism to explain RDL evolution compared to previous models that rely on system-specific mechanisms. For example, a model of the green alga *Volvox* predicts that RDL improves locomotory efficiency by enabling a more hydrodynamic body design. Such an explanation is unlikely to apply in general. In contrast, our model predicts that RDL is favoured whenever cheaters can prosper — a condition common across diverse biological systems and scales. This broad applicability makes our model a more general framework for understanding the evolution of RDL.



The conundrum of germline restricted chromosomes in black winged fungus gnats (Diptera)

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Most sciarids have germline restricted chromosomes (GRCs), which are present in germ cells but eliminated from somatic cells during embryogenesis in a conserved way. Other aspects of GRC evolution, however, seem less conserved. Notably, cytological evidence shows that the GRCs vary in size and number across the sciarid clade, and during meiosis GRCs have different patterns of transmission compared to other chromosomes in the genome and GRC transmission may vary in different individuals. We are currently working to understand, with both cytological and genomic analyses, how GRCs in sciarids evolved and how much GRC variation there is among and within species. So far, we have focused primarily on three sciarid species which can be kept in the lab: *B. coprophila* (2 GRCs), *B. impatiens* (1 GRC), and *Lycoriella ingenua* (2 GRCs). We generated chromosome level genomes for the germline of these species, and are complementing this dataset with additional genomic and

cytological evidence of GRC behaviour. In all three species, we see evidence for an origin of GRCs through introgression (likely via interspecific hybridization) from a gall gnat (Cecidomyiidae). Apart from evidence of their common origin, the five GRCs we sequenced lack large-scale gene conservation or synteny. This finding is intriguing for a number of reasons. We expected GRCs to carry some conserved genes, either for transmission or related to their germline location. However, we find that gene evolution on these chromosomes is extraordinarily dynamic, even between closely related species. Furthermore, GRCs are expected to recombine during female meiosis in species that carry two of these chromosomes. The fact that we sequence two distinct GRCs in species with two of these chromosomes suggests that these chromosomes may not recombine, suggesting a need to re-evaluate hypotheses of how these chromosomes are evolving.



The evolution of non-hybrid asexual reproduction in neotropical night lizards (*Lepidophyma* spp.)

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Reptiles are the only vertebrate clade in which true parthenogenesis, a form of asexual reproduction which does not involve male sperm, occurs. Many reptile species, including several birds, have been known to facultatively reproduce parthenogenetically. Although rare, most described all-female obligate parthenogenetic lineages are of hybrid origin, producing unreduced eggs using premeiotic endoreplication. Thus, all offspring are genetically identical to their mother. However, there is a unique vertebrate lineage where obligate parthenogenesis evolved without hybridisation - *Lepidophyma* night lizards from Central America. While most of the species in this genus are considered sexual, both facultative and obligate parthenogenesis occur in some species. Using both cytogenetic and genomic approaches, we studied the facultative parthenogenetic species *Lepidophyma smithii*, the sexual but regionally obligate

parthenogenetic *L. flavimaculatum* and the all-female *L. reticulatum*. We explore meiotic mechanisms and genomic heterozygosity underlying parthenogenesis in this genus. On top of that, we discovered diploid-triploid mosaicism in several individuals of *L. flavimaculatum*. We also assembled the genome of *L. reticulatum*, providing valuable insights into the evolution of parthenogenesis in vertebrates. We propose a novel mechanism where obligate parthenogenesis in *Lepidophyma* is not based on premeiotic endoreplication but instead evolved from the fixation of facultative parthenogenesis underpinned by fusion automixis, where an egg cell fuses with the polar body during meiosis. Altogether, we provide cytogenetic and genomic insights into the evolutionary mechanisms of the only non-hybrid origin of parthenogenesis in vertebrates.



When sex stops, genes change: ending sexual conflict alters gene expression

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Sexual conflict can arise because males and females, while sharing most of their genome, can have different phenotypic optima. Sexually dimorphic gene expression may help reduce conflict, but the expression of many genes may remain sub-optimal due to unresolved tensions between the sexes. Asexual lineages lack such conflict, making them relevant models for understanding the extent to which sexual conflict influences gene expression. We investigate the evolution of sexual conflict subsequent to sex loss by contrasting the gene expression patterns of sexual and asexual lineages in the pea aphid. Although asexual lineages of this aphid produce a small number of males in autumn, their mating opportunities are

limited because of geographic isolation between sexual and asexual lineages. Therefore, gene expression in parthenogenetic females of asexual lineages is no longer constrained by that of other morphs. We found that the expression of genes in males from asexual lineages tended towards the parthenogenetic female optimum, in agreement with theoretical predictions. Surprisingly, males and parthenogenetic females of asexual lineages over-expressed genes normally found in the ovaries and testes of sexual morphs. These changes in gene expression in asexual lineages may arise from the relaxation of selection or the dysregulation of gene networks otherwise used in sexual lineages.

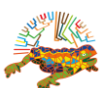




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S16 - Evolution in small populations



A century of isolation in contrasting environments drives evolutionary divergence in a small population of salmonid.

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POSTER SESSION 2 - **P02.063**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Small and isolated populations experience unique evolutionary dynamics shaped by selection, phenotypic plasticity, and potentially strong genetic drift. Understanding how multiple traits evolve in such populations is important for predicting their adaptive potential in response to climate change. Here, we investigate the divergence of metabolic, morphological and growth-related traits in two allopatric populations of Alpine charr (*Salvelinus umbla*) isolated for a century in ecologically contrasting lakes. We combined a common garden experiment with a quantitative genetic approach to disentangle the relative contributions of genetic divergence and plasticity to phenotypic differentiation. Population genetic differentiation was estimated using microsatellite markers and we applied Q_{st} - F_{st} comparisons to test whether phenotypic divergence resulted from adaptive (e.g., selection) or neutral (e.g., drift) processes. Our results revealed low neutral genetic differentiation ($F_{st} = 0.017$), suggesting limited drift, and Q_{st} - F_{st} comparisons indicated signatures of

divergent selection for several traits. Adults showed an important divergence in body shape and growth trajectories, potentially underlying a high environmental plasticity due to resource availability and temperature variation. In juveniles reared under common garden conditions, we found a substantial differentiation in metabolic rate, its allometric scaling and morphological traits likely related to trophic or visual functions (as indicated by an elevated Q_{st}), suggesting early-life divergent selection. These differences may reflect adaptation to distinct thermal regimes for metabolic traits, and to contrasting resource availability or foraging demands for morphological traits. This study provides empirical evidence of rapid trait evolutionary divergence in isolated populations, highlighting the role of both selection in evolutionary trajectories and phenotypic plasticity to phenotypic differentiation across environments. These findings highlight the importance of small, isolated populations in understanding adaptive potential in a changing climate.



A genomic toolkit reveals severe inbreeding depression in Seychelles warblers

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POSTER SESSION 2 - **P02.094**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Inbreeding threatens wild populations that have drastically shrunk. We test the effect of inbreeding on fitness in a recently bottlenecked population of Seychelles warblers (*Acrocephalus sechellensis*).

Materials and Methods: We have assembled the genomic toolkit for this species. This comprises a chromosome-level reference genome and millions of genome-wide markers acquired by low-coverage whole-genome sequencing followed by imputation, for around 2000 individuals. Genomic data accompanies three decades of detailed life-history traits measured from long-term monitoring of the island-enclosed population.

Results: With this data, we verify that the size and timing of the bottleneck coincides with European colonisation of Seychelles eroding

Ne to twelve, only nine generations ago. We find significant evidence of inbreeding depression, using genomic metrics of inbreeding and lifespan and lifetime fecundity as proxies of fitness.

Conclusions: Even island-living species, such as Seychelles warblers face inbreeding depression when population size shrinks. This suggests other wild populations may face the same and our genomic toolkit, and its scripts may help other researchers create their own toolkit to investigate this. In Seychelles warblers, we suggest evaluating the benefits of outcrossing with individuals from other island populations to prevent inbreeding depression. Our new genomic toolkit also allows for a range of other subsequent analyses including quantifying adaptive potential and loss of genetic diversity.



Behavioral adaptations at key genes in a small and isolated bear population facilitate coexistence with humans.

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POSTER SESSION 2 - P02.086, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The Apennine brown bear (*Ursus arctos marsicanus*) inhabits today a limited area in central Italy, where only around 50 bears survive in a human-dominated landscape. Once connected with the other European populations, it has been isolated for the last few thousand years, during which time direct persecution and land-use change caused a continuous population decline. This has led to marked genetic differentiation as well as morphological and ecological divergence. Interestingly, the Apennine brown bear is generally described as less aggressive compared to other populations, such as the European (*U. a. arctos*) and the North American brown bear (*U. a. horribilis*). While strong genetic drift has certainly shaped the genetic diversity of this endemic population, we hypothesized that prolonged environmental sharing with humans and direct persecution of the more aggressive bears may have driven the evolution of behavioral adaptations in the Apennine brown bear.

To test this hypothesis, we compared whole genome sequences of Apennine brown bears with bears from Slovakia, Alaska, and Canada as controls for larger populations with a documented history of aggression toward humans. Three complementary genome-scan methods were used to identify signatures of divergent selection in the Apennine brown bear. Additionally, in-silico analysis of highly differentiated variants was conducted to help elucidate the molecular mechanism possibly involved in the determination of a less aggressive phenotype.

We identified 566 candidate genes under selection in at least one of the genome scans. Functional enrichment analysis revealed a significant overrepresentation of genes associated with the human phenotype "overfriendliness" (seven outlier genes, $p=0.038$), suggesting a signal regarding behavioral mechanisms. Among the candidate genes, we identified ten additional genes known to influence behavior or neurological development in other species with tamer or domesticated forms.

The majority (14/17) of these behavior-associated genes showed highly differentiated intronic SNPs between the Apennine brown bear and the control populations, many of which are predicted to affect mRNA maturation via alternative splicing. In particular, one gene (SLC13A5) showed potential for exon skipping, and many genes were predicted to carry variation at splicing factor binding sites. This finding could imply that alternative splicing plays a role in determining the Apennine brown bear specific behavioral phenotype.

Our study suggests that the Apennine brown bear population has been subjected to human-mediated selection for a less aggressive behavior, likely through the historical elimination of the animals that were more prone to attack humans. Given the positive attitude of all the stakeholders sharing the land with this large mammal today, it is possible that this phenotypic trait has increased the chances of population persistence and human-bear coexistence.



Beyond panmixia: Disentangling structure and recent declines in a threatened humpback whale population using SFS- and LD-based demographic inferences

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POSTER SESSION 2 - P02.106, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Inferring the recent demographic history of a population from genetic data is a major challenge, particularly for wild species with poorly known population structure. This issue is exemplified in baleen whales, which experienced a drastic decline due to industrial whaling over the past two centuries. Among them, humpback whales (*Megaptera novaeangliae*) are estimated to have undergone a tenfold bottleneck over centuries of intensive whaling. Yet, the extent to which this recent change in census size has influenced the dynamics of genetic diversity remains unclear.

Several studies have attempted to quantify the genomic impact of whaling on different baleen whale populations, excluding humpback whales. However, these analyses often focus on a single sampling site and assume panmixia, a strong hypothesis that may lead to spurious demographic inferences when population structure is present. Detecting the consequences of whaling faces two major challenges. The first is to determine whether the population under study is better described by a panmictic or a structured model. The second is to successfully detect very recent demographic changes.

Here we assess the strengths and limitations of various computational tools to infer recent demographic events using the Madagascar humpback whale population as a case study. We use a large set of whole-genome re-sequenced individuals and extensive simulations to compare the results of demographic inferences based on either panmictic or structured scenarios, implementing either site frequency spectrum (SFS) based or linkage disequilibrium (LD) based inferential framework.

When applied to Malagasy humpback whale data, panmictic models reveal a strong bottleneck occurring only a few generations ago independently of the inferential framework used. These results are consistent with the historical records of whaling

and have been reported in another species, the fin whale, using SFS based approaches. However, when comparing structured vs panmictic models in a likelihood framework, SFS methods reveal that the former were statistically more supported than the latter, but did not recover a recent bottleneck. Conversely, LD-based modelling recovered a recent change in population size even in structured scenarios, yielding higher likelihood than panmictic models. To shed more light between the different outcomes of SFS vs LD modelling particularly concerning recent time variation, we performed a simulation-based study varying the time and intensity of the bottleneck in both panmictic and structured scenarios. A grid search over the two parameters suggested that LD-based inferences are more effective for recovering very recent signals (confirming hints from theoretical studies), and more capable of distinguishing changes in effective population size from changes in population structure.

In summary, SFS based approaches may overlook recent signature of population size change if only focusing on the likelihood distribution of different scenarios. This may have tremendous consequences especially in the field of conservation genomics. LD-based methods, still at their infancy when applied to non-model species, appear to be a promising alternative but need careful testing on a wider set of scenarios. Their validity should be also assessed when focusing on deeper temporal events. We believe that combining different inferential approaches will be warranted in the future to establish robust species-specific demographic scenario, as exemplified by our results on the humpback whale population from Madagascar. The correct understanding of the demography of a species is essential to predict the evolution of consanguinity and mutational load, which is of particular interest to improve the management of endangered population.



Colonisation costs drive weak purifying selection in island rails

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POSTER SESSION 2 - P02.072, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Insular (island-limited) populations typically show signatures of weak purifying selection, indicating small effective population sizes compared with those on the mainland. However, the source of these patterns is often unclear – they may be residual signatures from a brief period of inefficient selection associated with island colonisation, or they may reflect longer-term range limitations for insular species. Here we disentangle these hypotheses by analysing the drivers of variation in mitochondrial evolutionary rates (dN/dS) among 46 rails (Aves: Rallidae). We find that insular rails with short terminal branches (indicating recent island colonisation) have highly elevated mitochondrial dN/dS. In contrast,

rails representing more ancient island colonisations have dN/dS ratios that are indistinguishable from mainland/widespread species. Furthermore, we find that island size and the time since human settlement are unrelated to dN/dS ratio variation. Finally, we show that the elevated mitochondrial dN/dS of recently founded island rail populations is driven by generally weak purifying selection, as opposed to positive selection limited to a small number of sites. Taken together, these results indicate that insular rails suffer a high initial cost of island colonisation and undergo a period of inefficient selection, but that there is little detectable impact of longer-term range limitations.



Comparative Genomics in an Ex Situ Gorilla Population

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POSTER SESSION 2 - P02.105, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Conservation genomics has emerged as a crucial discipline for understanding and preserving biodiversity, especially for critically endangered species such as gorillas. Gorillas are classified into two distinct species: western and eastern. Each species is subdivided into two subspecies: western gorillas into the western lowland and Cross River gorillas, and eastern gorillas into the eastern lowland and mountain gorillas. All four subspecies are labeled as endangered (mountain gorillas) or critically endangered (western lowland, eastern lowland, and Cross River) by the IUCN.

We analyze 248 gorilla whole genomes (93 publically available, and 155 newly sequenced). Of the 248 genomes, 205 belong to the captive western lowland gorillas, the main captive population studied in this project, including 169 individuals born in captivity and 36 born in the wild. We also include 1 captive Cross River and 2 captive eastern lowland individuals. Moreover, a small subset of the 248 genomes belongs to wild individuals from the eastern lowland (10 individuals), Cross River (18 individuals), and mountain gorilla (12 individuals) subspecies, included for comparative genomic purposes.

In this work we illustrate patterns of ancestry, genetic diversity, relatedness, and inbreeding of captive living gorillas housed primarily in European Zoos, to add information that could help in conservation strategies. Furthermore, we analyze the relatives of “Floquet de Neu” gorilla to determine which ones carry the autosomal-recessive mutation responsible for Floquet’s albinism.

Whole genome samples in this study are obtained from various sources, including whole blood, hair, and blood stored on FTA cards. Whole blood samples exhibit higher levels of endogenous DNA compared to the other types. Additionally, genomes derived from blood

samples show significantly higher mean coverage (~22X) than those from hair and FTA cards (~10X). This variation in coverage is accounted for in downstream analyses.

The findings reveal distinct clustering between western and eastern gorilla species, as seen in previous studies, with evidence of admixture among western lowland individuals born in captivity. This admixture likely results from breeding between individuals from the same subspecies but originating from geographically distant wild populations (e.g., different countries).

There is a noteworthy degree of relatedness observed among the analyzed captive population of Western Lowland gorillas (a total of 179 pairs of first-degree relatives are found within the western lowland captive population). Moreover, the predicted heterozygosity value of the analyzed western lowland individuals is higher compared to those of the examined eastern lowland and mountain gorillas, in agreement with previous studies. Finally, when discussing the DNA of Floquet’s relatives, seven individuals are found to carry, in heterozygosity, the mutation responsible for Floquet’s albinism.

The implications of this study for the EEP (European Endangered Species Programme) provide new insights into the specific genetic information of all captive living gorillas in European zoos.

Additionally, clarifying the degrees of relatedness among these individuals is valuable for future comparisons and updates to the current Studbook—a comprehensive record tracking the genetic lineage, demographics, and history of each individual gorilla. This clarification will support the planning of effective breeding programs to reduce inbreeding within the population.



Conservation genomics in action: Advancing conservation of the small populations of threatened Australian flora

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POSTER SESSION 2 - **P02.091**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Flora in Australia is highly diverse yet exists within highly fragmented landscapes. Native plant populations face numerous threats, including from urbanisation, altered fire regimes, disease, and invasive weeds and pests. While various conservation efforts are in place, significant gaps in species-specific knowledge hinder development and implementation of effective conservation strategies.

Genomic technologies are transforming plant conservation by offering crucial insights into taxonomic resolution, genetic diversity, population viability and evidence-based management strategies. Streamlined workflows make the integration of genomics into conservation programs feasible, and the growing body of genomic studies highlights resulting practical applications, including ensuring limited conservation resources are allocated properly to maximise conservation outcomes at minimal cost.

At the Botanic Gardens of Sydney, a highly applicable conservation genomics workflow is supporting the conservation of over a quarter of threatened plant species in New South Wales, Australia. This includes a range of species facing different threats and requiring tailored conservation approaches.

The workflow is designed to address target questions to empower threatened species officers to make informed, evidence-based decisions on conservation strategies aimed at supporting long-term species survival.

To illustrate the utility of this workflow, we present genomic findings on a selection of species with varying conservation status and causes of decline. These examples demonstrate diverse processes influencing small, vulnerable populations:

- *Astrotricha crassifolia* - a species with two highly disjunct small populations, each consist of plants of varying growth habits
- *Eidothea hardeniana* - a narrow endemic species restricted to a single location, recently impacted by altered fire regimes
- *Rhodamnia rubescens* – formerly widespread but now severely impacted by Myrtle Rust

These case studies exemplify how genomics can inform and tailor management interventions, ultimately enhancing the effectiveness of conservation planning across diverse ecological contexts.



Differentiation in host plant performance among beetle populations – adaptation to local plant community?

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POSTER SESSION 2 - **P02.087**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Host plants are essential for the fitness of phytophagous insects, and spatial variation in host plant availability may lead to local variation among populations through adaptive evolution or phenotypic plasticity. Studying intraspecific variation in host plant adaptation can give us insights into local degrees of specialization and potential for adaptability to environmental changes, such as shifts in host plant community.

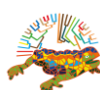
The green tortoise beetle *Cassida viridis* is a phytophagous specialist that utilizes a few plant species within the Lamiaceae family as hosts. The host plants are vital to the beetle's life cycle since they mate, oviposit, and feed at the host plants. Therefore, the distribution of plants restricts *C. viridis* to small patches in the heterogeneous landscape. We studied how host plant performance varied in four Swedish *C. viridis* populations, which differ in host plant compositions ranging from a single plant species to a mixture of species. Two of the populations sampled are located 30 km apart in Skåne, southern Sweden, while the other two are situated in Halland and Närke, approximately 200 km and 300 km north of the Skåne populations, respectively.

We collected oothecas (egg capsules) from the field and installed them in controlled lab

conditions. After hatching, individual larvae from each oothecum were transferred into separate petri dishes with either mint *Mentha* spp, gypsywort *Lycopus europeus* or hempnettle *Galeopsis tetrahit* to feed on. We recorded development time, pupae weight and survival. Pictures were taken of the adult beetles for morphological image analysis.

Preliminary data indicate differences in performance between and within populations. It seems that beetles from Skåne have a much lower probability of surviving on hempnettle compared to the populations in Halland and Närke. This difference between populations may be a result of adaptation to the local host plant community as the populations in Skåne do not contain hempnettle, while the population in Närke exclusively have hempnettle as host species, however the population in Halland neither contain hempnettle, only mint as host.

These findings can give us insight into local specialization patterns of phytophagous insects and how strength of selection from different host plant species varies among populations.



DNA methylation: a way for fast-renewed inbreeding depression in animals? An example with the mollusc species *Physa Acuta*.

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POSTER SESSION 2 - **P02.062**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Inbreeding depression (ID), defined as the reduction in fitness of offspring resulting from matings between related individuals compared to random individuals, has major effects expected on endangered species with small and/or declining populations, but also on evolution of the mating system. Usually, the main factor mentioned to explain ID is the loss of genetic diversity that compromise the evolutionary response to environmental change and the expression of recessive deleterious genetic mutations (named also genetic load) in inbred lines. However, this theory does not include an important factor that could also modify fitness of individuals, the epigenetic variants (or epi-alleles).

Despite the effects of DNA methylation and other epigenetic modifications on phenotypes, and the recurrent demonstrations of their contributions on rapid adaptation to environmental in different species, the potential negative effect of epigenome on individual fitness was not considered yet. Among all epigenetic marks, DNA methylation has received the most attention in evolutionary studies because of its pivotal role in repressing the mobilisation of transposable elements and gene expression, and of its potential inheritance. Moreover, DNA methylation variation arises at a considerably higher rate than genetic mutations in response to genetic, environmental and stochastic factors, creating fastly new advantageous or disadvantageous phenotypes.

Thus, epi-alleles, as genetic mutations, meet all the criteria required to promote ID in small populations: they can vary within and

between natural populations, be inherited across generations, and influence individual fitness. This assumption remains, however, to be tested in natural populations. Demonstrating the existence and understanding the evolution of this “epigenetic load” from an empirical point of view is essential to complete theoretically the expectations about ID in small populations.

Experimentally, to test this hypothesis requires distinguishing the contribution of the epigenetic to the overall ID by comparing the fitness of offspring from a series of controlled autogamous or allogamous crosses, but between individuals highly genetically inbred which deleterious genetic mutations were supposed highly purged.

Physa acuta is a short-generation hermaphrodite organism easy to breed in the laboratory, with a high level of ID in natural populations and that can self-fertilize when no partner is available. Using the progeny of the F1 obtained after crosses of two highly inbred lineages of this species, obtained after 28 generations of selfing, we demonstrated that fast new ID exist in offspring obtained by selfcross compared to cousin-cross. Then, using the phased haplotype of methylation obtained by Nanopore technology of grand-parents, parents and offsprings from the different type of crosses, cumulating with epiGBS data, we proposed an experimental protocol to estimate the component of ID attributed to the epi-alleles, their inheritance and identified this “epigenetic load”.



Drivers of adaptive evolution in a relict brown bear population

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POSTER SESSION 2 - **P02.098**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The evolutionary dynamics of small, isolated populations provide valuable insights into how genetic constraints interact with environmental factors to drive adaptive phenotypic shifts. The Apennine brown bear (*Ursus arctos marsicanus*), one of the most endangered, isolated and genetically depleted bear populations in Europe, exhibits striking phenotypic divergence from its conspecifics. Particularly, the rapid emergence of distinct cranial features, such as enlarged zygomatic arches and unique molar morphology, is likely associated with shifts toward a more herbivorous diet and may represent adaptive responses to the specific ecological context of the Apennines.

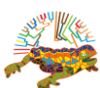
Here, we present the results of ten years of research on the evolution of this iconic relict population. This study provides valuable data on the mechanisms underlying phenotypic evolution in small, isolated populations.

Analyses of skull shape conducted on Apennine, Scandinavian, and Kamchatka brown bear populations using 3D geometric morphometrics suggested a reduction in morphological integration, i.e. the coordinated variation of traits due to genetic and epigenetic interactions, as a possible facilitator of increased phenotypic evolvability in the Apennine population. In particular, the altered trait covariance patterns in the in skull shape suggest that the relaxation of developmental constraints may have unlocked novel morphological trajectories. Hence, the observed differences of the Apennine brown bear may reflect adaptive evolution fostered by developmental plasticity rather than neutral processes alone. Although this population has recently been the subject of genomic studies, the molecular mechanisms underlying this divergence remain poorly understood. To gain additional insight into the microevolutionary processes acting at the population level, the potential role of epigenetic regulation in the evolution of this population was investigated by performing a

genome-wide methylation analysis on three European brown bear populations, Apennine, Alpine, and Scandinavian. Using Reduced Representation Bisulfite Sequencing (RRBS), population-specific epigenomic signatures were identified. The methylation profiles revealed marked population-level differences, highlighting epigenetic divergence as a potential driver of population differentiation. Around 50% of the differentially methylated regions (DMRs) were found both in promoters and non-coding areas distal from transcription start sites, suggesting that regulatory shifts, rather than single nucleotide mutations, may play a pivotal role. Gene Ontology analysis linked these DMRs to genes involved in anatomical structure development, morphogenesis, and immune function.

These epigenomic findings align with geometric morphometric analyses that reveal reduced morphological integration and increased fluctuating asymmetry, both considered hallmarks of altered developmental pathways. We propose that isolation and reduced genomic diversity, combined with epigenetically mediated regulatory changes, have contributed to the emergence of unique phenotypic traits in the Apennine brown bear. This case study may exemplify how developmental modifications, driven by changes in regulatory networks, can enhance phenotypic evolvability and promote adaptive divergence in small, isolated populations.

Our findings support an integrative evolutionary framework that combines epigenomics and morphometrics. This approach advances our understanding of how phenotypic novelties can emerge rapidly in small populations and underscores the importance of non-genetic mechanisms in shaping biodiversity. Such integrative insights are essential not only for evolutionary biology but also for informing the conservation of genetically impoverished yet phenotypically distinct lineages.



Ecological genetics of isolated loach populations indicate compromised adaptive potential

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POSTER SESSION 2 - **P02.075**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Many endangered species live in fragmented and isolated populations with low genetic variability, signs of inbreeding, and small effective population sizes - all features elevating their extinction risk. The flat-headed loach (*Oreonectes platycephalus*), a small noemacheilid fish, is widely across southern China, but only in the headwaters of hillstreams; as a result, they are spatially isolated from conspecific populations. We surveyed single nucleotide polymorphisms in 16 Hong Kong populations of *O. platycephalus* to determine whether loach populations from different streams were genetically isolated from each other, showed low levels of genetic diversity, signs of inbreeding, and had small contemporary effective population sizes. Estimates of average observed heterozygosity ($H_o = 0.0473$), average weighted nucleotide diversity ($\pi_w = 0.0546$) and contemporary effective population sizes ($N_e = 10.2 \sim 129.8$)

were very low, and several populations showed clear signs of inbreeding as judged from relatedness estimates. The degree of genetic differentiation among populations was very high (average $F_{st} = 0.668$), even over short geographic distances (<1.5 km), with clear patterns of isolation by distance. These results suggest that Hong Kong populations of *O. platycephalus* have experienced strong genetic drift and loss of genetic variability because sea-level rise after the last glaciation reduced connectedness among paleodrainages, isolating populations in headwaters. All this, together with the fact that the levels of genetic diversity and contemporary effective population sizes within *O. platycephalus* populations are lower than most other freshwater fishes, suggests that they face high local extinction risk and have limited capacity for future adaptation.



Effects of long-term inbreeding on assortative mating preferences and dynamic colour-ornament expression in a cichlid fish

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POSTER SESSION 2 - **P02.067**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

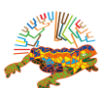
Background: The structuring of populations – due to natural or anthropogenic causes – can have important evolutionary implications. The resulting subpopulations are often small and prone to inbreeding. Inbreeding can lead to inbreeding depression, which severely affects fitness-related traits and can lead to population extinction. However, in combination with genetic drift, continuous inbreeding can lead to random genetic differences between subpopulations, which could contribute to population divergence and, thus, could drive diversification. In addition, purging processes associated with inbreeding can remove recessive deleterious alleles with large or medium effects from the population. Nevertheless, inbreeding depression may become apparent in polygenic, condition-dependent traits.

Methods: Here, we investigated the effects of long-term inbreeding on assortative mating and dynamic color expression during intra-sexual competition in *Pelvicachromis taeniatus*. This African cichlid fish shows intense biparental brood care and pronounced kin mating preferences. Both sexes display colourful ornaments that influence inter- and intra-sexual selection. Previous population genetic studies have shown that the natural population is inbred and substructured. Laboratory experiments revealed benefits from kin mating, as breeding pairs consisting of related individuals (full siblings) were more cooperative and less aggressive among each other than pairs consisting of unrelated individuals, as predicted by kin selection theory. Here, we conducted dichotomous mate choice experiments in which inbred and outbred individuals were given the choice between their own species vs. a foreign species, and kin vs. non-kin. Reinforced assortative mating preferences in

inbred groups could lead to divergent tendencies. In contrast, inbreeding depression could result in poorer performance of inbred fish compared to outbred fish. We also compared the effect of inbreeding on colour ornamentation in both sexes and the ability of inbred and outbred males to maintain intense body colouration during competition for breeding sites. Males show carotenoid-based yellow coloration in the fin and the ventral body region. Yellow intensity is condition-dependent and associated with dominance behaviour.

Results: Highly inbred individuals showed similar assortative mating preferences for the own species as outbred individuals. In terms of kin-mating preferences, outbred fish showed similar inbreeding preferences as in previous studies, whereas highly inbred fish preferred unrelated mating partners from a different inbred line to related partners suggesting that inbredness triggers outbreeding preferences. Inbreeding also affected the intensity of male colour ornaments with inbred males being less intensely coloured than outbred males after competing with rivals.

Conclusions: Our results indicate that persistent inbreeding does not reinforce assortative mating preferences and, thus, does not appear facilitate population divergence. In contrast, inbreeding induces disassortative preferences with respect to the genetic relatedness of the partner, i.e. highly inbred individuals showed inbreeding avoidance. This suggests an oscillation between inbreeding preference and inbreeding avoidance depending on the level of inbredness. Inbreeding depression in ornamental traits only became apparent in a competitive environment suggesting environment-dependent inbreeding depression.



Estimating realized relatedness in a wild baboon population and its implications for kin-biased behavior

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POSTER SESSION 2 - P02.058, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Kinship is a major determinant of affiliative and mating behaviors in many animal species. While animals are often capable of discriminating between closely related individuals, the underlying mechanisms of kin discrimination typically remain unclear, especially in natural populations. This challenge stems in part from the problem that relatedness estimates based on pedigrees from natural populations are imperfect. Recent methods to infer the length and number of segments identical-by-descent (IBD) across the genomes of pairs of individuals can help overcome this problem, because IBD can be used to identify kin that are cryptic to observers, the animals themselves, or both. Here, we use genome-wide data from 433 wild baboons to estimate IBD-based realized relatedness within the intensively studied Amboseli population in Kenya. First, we demonstrate that IBD segments can be identified with high accuracy using genome resequencing data with as low as ~1x coverage paired with imputation. By using the resulting IBD calls, we both more precisely measure the actual

biological relatedness between known relatives (based on an independently constructed multigenerational pedigree) and identify new pedigree links that improve the match between pedigree and IBD-based relatedness estimates. In the process, we identified 17,396 cryptic relatives who were previously missed by the pedigree, but where IBD calls suggest third-degree relatedness or closer. Among these, 614 cases involve opposite-sex pairs of baboons who consorted as adults, and who were unlikely to identify each other as kin based on familiarity during development because they were born and matured in two different social groups. In 51 of these 614 cases, the pair produced inbred offspring. These results suggest that dispersal is a double-edged sword in our population—it frequently acts as a barrier to inbreeding by separating opposite-sex adult kin, but it sometimes introduces unfamiliar kin to one another in adulthood. Our findings thus lay important groundwork for understanding how and when kin discrimination in the context of mate choice evolves in the wild.



Evaluating genotype imputation in the Iberian lynx: a conservation genomics approach for small and endangered populations

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POSTER SESSION 2 - **P02.101**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Small and isolated populations are particularly vulnerable to genetic erosion due to a reduced effective population size, increased drift, and inbreeding. The Iberian lynx (*Lynx pardinus*) experienced such severe bottlenecks that, by 2002, only ~100 individuals remained, and they were divided into two differentiated populations: Doñana and Sierra Morena. Thanks to several conservation actions, including captive breeding, translocations and reintroductions, populations are experiencing a remarkable recovery.

While high-coverage whole-genome sequencing (WGS) can provide valuable insights into evolutionary processes, it remains costly for large-scale studies. Low-coverage WGS (lcWGS) followed by genotype imputation offers a cost-effective alternative to characterize genome-wide variation in threatened species. Here, we assess the reliability of imputed genomic data for estimating key genetic parameters in the Iberian lynx, such as population structure, demographic history, and patterns of inbreeding and ancestry. **Materials and Method:** We downsampled high coverage (~25X) WGS data from 50 individuals of Iberian lynx to simulate lcWGS datasets at depths ranging from 0.1X to 2X. These individuals represented both remnant populations and admixed individuals and were used as a reference panel for imputation using GLIMPSEv1.1, following a leave-one-out validation approach. To assess scalability, we simulated a multi-target imputation scenario incorporating additional WGS data to be imputed from 150 individuals sequenced at ~1X, 19 at ~6X, and 11 at ~25X from previous studies.

Imputation accuracy was evaluated by comparing inferred genotypes under different quality filtering thresholds to those obtained from high-coverage data. We then reconstructed the demographic history of the species using

GONE, and estimated global (FROH, ADMIXTURE) and local genomic patterns of inbreeding and ancestry by analysing the overlap between runs of homozygosity (ROH) and ancestry-specific tracts in both high-coverage and imputed datasets.

Results: Imputation accuracy was generally high, increasing with coverage and varying among populations. The Doñana population, with lower genetic diversity and longer shared haplotypes, showed higher imputation accuracy overall, though heterozygous sites were less reliably recovered. The inclusion of additional samples in the multi-target imputation scenario significantly improved genotype concordance, particularly for the lower coverage datasets.

Global estimates of inbreeding and ancestry were largely consistent between high-coverage and imputed datasets. Nonetheless, local-scale patterns showed greater variation: longer ROH and recent ancestry tracts were recovered with higher accuracy than shorter segments which, in the case of ROH, were often overestimated. Similarly, GONE reliably inferred recent demographic events but produced less consistent results for older time periods.

Conclusions: Genotype imputation from lcWGS offers a reliable, scalable tool for evolutionary and conservation genomics in small and endangered populations. This strategy can recover reliable information on recent inbreeding, admixture, and demographic changes, particularly in populations with low genetic diversity. However, precision declines for older or finer-scale genomic features. Importantly, performing a multi-target imputation with as many WGS data available can significantly enhance imputation performance. For the Iberian lynx, this approach will be used to investigate recent evolutionary dynamics and informing ongoing conservation management.



Evolutionary dynamics of invasive *Vespula germanica*: The role of dispersal and climate in a restricted population

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POSTER SESSION 2 - **P02.056**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Biological invasions drive rapid evolutionary change, challenging native biodiversity and ecosystem stability. They are a prime example of how populations founded by a few individuals expand and evolve in new environments. In South Africa, the European wasp *Vespula germanica* (Hymenoptera: Vespidae) established itself in the early 1970s and remains geographically constrained compared to other invaded world regions. This provides a unique opportunity to study the evolutionary dynamics of an isolated, slowly expanding population. We analyzed the genomes of 50 wasps representing the South African distribution using 2b-RAD sequencing. Our results revealed two main genetic clusters, each corresponding to a geographical region, which align with previously identified mitochondrial haplotypes and their distribution. This population structure supports the hypothesis of double

introduction and suggests limited male dispersal. Interestingly, the absence of isolation by distance patterns implies that gyne dispersal, whether natural or human-mediated, plays a significant role in maintaining genetic homogeneity within each cluster. Furthermore, we found an association between temperature and genetic structuring, with individuals from one cluster inhabiting warmer regions. This correlation, evident at both mitochondrial and nuclear levels, supports the hypothesis of climate-driven selective pressures shaping population structure and provides insights into the evolutionary processes occurring in small populations. This study represents the first genomic analysis of *V. germanica* and contributes to our understanding of population genomics and ecological dynamics in restricted populations of invasive wasps.



Evolutionary potential for pollutant tolerance in urban *Daphnia*

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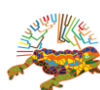
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POSTER SESSION 2 - P02.084, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Due to a steady projected growth of cities in the near future and their associated stress and health risks for ecosystems and people, urbanisation has become a key aspect of global change. Urban ponds provide important ecosystem services in cities, but their functionality is threatened by influx of urbanisation-associated pollutants from different sources, such as urban stormwater runoff. Among these, synthetic organic chemicals - including those associated with traffic, such as tire wear chemicals - pose a growing threat to the ecological integrity of urban ponds. As urban ponds play a key role in mitigating climate impacts in cities, understanding how their biological communities respond to pollution is critical. Recently *Daphnia* have been used as a promising model system to study urban evolution. Their ability to top-down control phytoplankton blooms highlights their importance as key interactors in urban pond ecosystems. Urban stressors were previously shown to affect *Daphnia* fitness and species composition, potentially impacting their grazing capacities. Yet, little is known about how *D. magna* populations cope with increased pollution loads in urban ponds and whether populations differ in their

evolutionary potential to adapt to the emerging threat of urban pollution.

In this study, we exposed *D. magna* clones derived from 5 urban and 5 rural populations in the Berlin-Brandenburg area to the tire wear chemical 1,3-Diphenylguanidine (DPG). DPG is associated with traffic intensity and was recently shown to negatively affect another waterflea - *Moina macrocopa*. In a hierarchical design with clones nested in populations nested in level of urbanization, we analysed acute sensitivity of *D. magna* clones to evaluate to what extent there is genetic variation in tolerance within populations, whether populations differ in their tolerance and whether this is linked to urbanisation level. Our results point to substantial genetic variation in DPG tolerance across clones and populations, showing strong potential for evolutionary responses to changing pollution levels. Yet, we did not find an association between higher DPG tolerances and higher levels of urbanization, nor between DPG tolerance and measured DPG concentrations in the field. Nevertheless, our study provides insights into how rapid adaptation may shape future responses to anthropogenic environmental change.



Evolutionary rescue in small populations: latitudinal variation in wing polyphenism modifies genetic diversity and connectivity across natural populations in a water strider.

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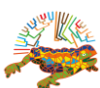
POSTER SESSION 2 - P02.079, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Species that occur in fragmented habitats and at small population sizes are at most risk of genetic erosion and local extinction, particularly during rapid environmental changes. Studying the evolutionary dynamics of small populations at both the phenotypic and genetic level is therefore of critical importance but remains difficult as these species are often rare and threatened. We use a natural study system of a species of water striders that occur in rivers and are widespread across Europe to examine how changes in an environmentally-induced phenotype (wing polyphenism) impact different evolutionary processes. *Aquarius najas* displays a latitudinal cline in wing frequency across Europe, with polymorphic populations in the South and wingless ones in the North. Hence, it represents an ideal system to study the interaction between a phenotypically plastic trait and how this alters genetic diversity and thus microevolutionary processes that impact survival of populations.

Materials and methods: To understand the population genetic consequences of wing polyphenism we first assembled a reference genome combining PacBio HiFi and Dovetail Omni-C with ISO-seq for annotation. To obtain estimates of genetic variation and differentiation we sampled multiple populations across Europe for sequencing (ddRAD/ low coverage WGS), focusing particularly on populations in Spain and Finland as they are located at the opposite ends of the latitudinal cline in wing frequency. We measured genetic diversity and inbreeding in each site, as well as differentiation between them. We reconstructed the demographic history of the species, obtaining both current and historical estimates of effective population size and we also reconstructed the species ecological niche at different timepoints to provide insights of the recolonisation of Europe after the Last Glacial Maximum and potential future response to climate change.

Results: The reference genome included 12 highly-contiguous chromosomes (L = 886Mbp, N = 73.5Mbp, 52% repeat content). Population genetic analyses for ca. 600 specimens revealed strong geographic clustering across Europe with 11 different lineages. These were mostly geographically restricted, although multiple coexisting lineages were detected in Spain and Finland. Country-specific structure analyses strongly suggest a higher connectivity across sites in Spain than in Finland, as expected given the higher frequency of winged individuals in Spain. These patterns are confirmed by measures of diversity, which are ca. 4-fold higher in Southern latitudes, as well as inbreeding and differentiation, which are low in Spain but extremely high ($F_{ST} > 0.65$ between sites ~20 km apart) in Finland. Measurements of effective population size indicate extremely fragmented populations in the North ($25 < N_e < 150$) and differ geographically.

Conclusions: Our study highlights the extreme fragmentation of natural populations of *Aquarius najas* and how a phenotypically plastic trait can dramatically alter fundamental evolutionary processes and the distribution of genetic variation. The species is subject to different evolutionary pressures with Southern populations exposed to great seasonal and yearly variability in their habitats due to a increased risk of habitats drying out because of climate change. In contrast, habitat stability of Northern populations is very high but populations are highly inbred and thus prone to genetic erosion as a result of low connectivity due to a lack of migrants. We demonstrate how important phenotypic plasticity can be in modulating dispersal and thus help determine microevolutionary patterns in a species that occur in a fragmented landscape at small population sizes.



Exploring the effects of first year cold-dormancy on the phenotypic, molecular and metabolic responses in head-started Gopher tortoises (*Gopherus polyphemus*)

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POSTER SESSION 2 - **P02.090**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Head-starting programs are increasingly used in reptile conservation to improve early survival by promoting expedited growth during early vulnerable juvenile stages. However, in species like the gopher tortoise (*Gopherus polyphemus*), endemic to the Southeast USA, the head-starting environment that promotes fast growth is often a dramatic shift from what those animals would be experiencing in the wild in their first year of life in its natural populations. Therefore, there is a significant gap in knowledge regarding how bypassing natural cold dormancy early in life impacts essential biological functions including growth trajectories, metabolic processes, and molecular responses in gopher tortoises that may impact fitness. In this study, we experimentally test the effects of bypassing first-year cold dormancy on growth rate, telomere length, mitochondrial DNA (mtDNA) density, and metabolic markers in head-started tortoises. We used a split-clutch design to assign 60 hatchlings from 14 clutches to two treatment groups: (1) a constant-warmth group raised under standard head-starting conditions without dormancy, and (2) a cold-dormancy group housed under simulated Alabama winter conditions. We took monthly body measurements and collected blood and plasma at four timepoints: before-dormancy (before), during-dormancy (during), 3 weeks post-dormancy (3Wk.Post) and 3 months post-dormancy (3Mo.Post). We estimated growth rate across timepoints, used qPCR to estimate mtDNA density and telomere length, and quantified glucose, total triglycerides and acetyl-CoA in blood plasma. We found that animals that experienced cold-dormancy were 8.47 mm smaller at 3Mo.Post dormancy relative to the constant-warmth group. This was due to the cold-dormancy group having a growth rate 0.54 g/day lower than the constant-warmth group during dormancy, and 0.51 g/day lower in the three weeks after coming out of dormancy (both results were

statistically significant). However, 3Mo.Post dormancy there was no statistically significant difference in growth rate between treatment groups. This indicates that although the cold-dormancy animals were smaller, they were able to resume the same growth rate as the constant-warmth group by 3Mo.Post dormancy but not the same body size. Additionally, there was no effect of either growth rate or treatment on telomere length across timepoints. However, while there was no difference in mtDNA density between the two treatment groups during dormancy, we found that the cold-dormancy group showed a significant 27.1% increase in mtDNA density at 3Wk.Post dormancy followed by a significant 17.1% decrease compared to the constant-warmth group 3 Mo.Post dormancy. Furthermore, during cold-dormancy tortoises had reduced plasma glucose compared to the constant-warmth treatment, but there were no differences at post-dormancy timepoints. Finally, our results showed that experiencing cold-dormancy caused a significant decrease in both total triglycerides and acetyl-CoA at 3Wk.Post dormancy compared to the constant-warmth. Total triglycerides at 3Mo.Post were not significant (acetyl-CoA was not measured). Our findings suggest that the period of growth and metabolic suppression associated with cold dormancy persists for at least 3 weeks after dormancy but is reversible by 3 months after dormancy. While telomere length might be maintained through compensatory cellular mechanisms during early life stage, the metabolic responses could influence energetic priorities and recovery processes associated with arousal from dormancy. These results have important implications for conservation management, suggesting that supporting the growth and metabolic health of reintroduced gopher tortoises may require greater attention to their natural physiological rhythms.



Exploring the Genomic Signatures of Convergent Dietary Specialization in the Adaptive Radiation of Canary Island Dysdera Spiders

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POSTER SESSION 2 - P02.080, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Island radiations offer a powerful framework for investigating the molecular basis of evolutionary processes and evaluating their predictability. In archipelagos, the presence of multiple independent evolutionary replicates provides a unique opportunity to test for molecular parallelism underlying phenotypic convergence. Here, we study the remarkable diversification of the spider genus *Dysdera* in the oceanic archipelago of the Canary Islands, which has given rise to approximately 60 endemic species over the past 20 million years. This radiation has been accompanied by dietary shifts, including morphological, metabolic and behavioural specialisations for feeding on woodlice (Isopoda), which bioaccumulate exceptionally high concentrations of heavy metals from the soil. Hence, these endemic spiders represent an excellent model for investigating the evolutionary drivers and genomic factors underlying dietary convergence and adaptive radiations.

To understand the genomic basis of this specialization at short- and mid-term evolutionary timescales, we relied on a dual

approach integrating population and comparative genomic analyses. To this end, we generated high-quality chromosome-level genome-assemblies and performed low-coverage whole-genome resequencing of multiple individuals from natural populations of five endemic *Dysdera* species from the Canary Islands. The selected species were chosen to capture two independent shifts of dietary specialisation, each occurring in distinct evolutionary and geographic contexts. We uncovered exceptionally high levels of nucleotide diversity in all five species, indicative of large historical effective population sizes. In addition, we identified several candidate genomic regions that show signatures of recent selective sweeps, highlighting potential targets of adaptation. Furthermore, we are exploring whether changes in selective constraints are associated with dietary specialisation. This study advances our understanding of the genomic basis and the evolutionary forces shaping convergent evolution and adaptive radiation in island ecosystems.



First Insights into Population Structure of the Endangered Darwin's Fox (*Lycalopex fulvipes*).

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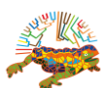
POSTER SESSION 2 - **P02.077**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Darwin's fox (*Lycalopex fulvipes*) is currently the most endangered canid in South America, with less than 1,000 individuals surviving in the highly fragmented Valdivian temperate rainforest, an internationally recognized biodiversity hotspot in southern Chile. This small, forest-specialized endemic fox is threatened by deforestation and other anthropogenic activities, persisting in isolated native forest remnants. Despite its importance as a flagship species for the conservation of the Valdivian temperate rainforest, no population genetic studies have yet assessed the species across its full geographic range.

Materials and Methods: We reconstructed complete mitochondrial genomes for 37 Darwin's fox individuals spanning the species' current known geographic range, using mostly low-to-medium coverage whole-genome resequencing data (35 new, 2 previously published). This constitutes the first population-scale study of Darwin's fox using high-throughput sequencing. Phylogenetic relationships were reconstructed using median-joining, maximum likelihood, and Bayesian inference.

Results: We found differentiation between the two main distribution areas, Chiloé Island and the Chilean mainland, as well as further substructure among the latter. We observed a low number of haplotypes despite employing complete mitochondrial sequences, with high incidence of haplotype-sharing within localities, and high sequence divergence between geographic regions (incl. mainland localities).

Conclusions: This study addresses a major knowledge gap regarding intraspecific partitioning of genetic variation in Darwin's fox, providing the first insights into population structure and history of this endangered species. The observed distribution of intraspecific variation suggests restricted female-mediated gene flow among sampled localities. Additionally, molecular dating provides new information regarding the colonization of Chiloé Island by Darwin's foxes. These preliminary findings may be used to generate diagnostic mtDNA-variants for population assignment, thus benefiting evidence-based conservation planning.



From hives to genomes: inferring human impacts and genomic consequences of evolution in small bee populations

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POSTER SESSION 2 - **P02.071**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Western honey bees (*Apis mellifera*) are essential pollinators, playing a key role in both biodiversity and agriculture. In Europe, traditional beekeeping - based on capturing wild swarms - has gradually been replaced by more modern practices, including raising queens, selecting high-performing lines, and using movable-frame hives. Although honey bees remain mostly wild, these changes explain why they are often considered a semi-domesticated species. After World War II, and especially since the 1980s, modern beekeeping has induced large-scale movement and mixing of honey bee subspecies. This has led to a reduction in the use of local subspecies (such as the black honey bee in Western Europe) and promoted artificial selection on others (e.g. *A. m. ligustica*, *A. m. carnica*, and Buckfast).

We analyzed hundreds of honey bee genomes to better understand their recent evolutionary history and how it affects current genetic diversity. Using Approximate Bayesian Computation, we found evidence of postglacial expansions and secondary contact between lineages before human influence. These natural processes were followed by more recent human-mediated movements.

To assess changes in population size over time, we used the program GONE. This revealed strong bottlenecks over the past 25 generations (~50 years) across many subspecies and breeds. Some exhibited gradual declines in GONE-inferred N_e which is likely due to selective breeding practices. These were especially strong in black honey

bee populations kept on small islands, where conservation programs have been set up to limit introgression from non-native bees. Indeed, these islands are often far enough from the mainland to prevent cross-mating, which helps preserve the native genetic background, but are limited in space and hive numbers. We analysed bees from two islands and inferred that both populations experienced a severe bottleneck when founded, and were maintained at very low effective population sizes ($N_e \sim 100$).

Among populations with low N_e values, we observed reduced genetic diversity, which may lower the bees' ability to adapt to environmental stress or disease, raising concerns about long-term sustainability of isolated conservation strategies. We also detected high levels of inbreeding and a moderate increase in the load of deleterious mutations, especially in regions of low recombination. This fits with the nearly neutral theory. The load is however likely limited by the fact that honey bees are haplodiploid, consequently recessive deleterious mutations in haploid males are more effectively removed from the population than in purely diploid systems.

Our study highlights that conservation programs, especially on islands, must consider genetic diversity and long-term viability. To achieve this, public funding for conservatories to facilitate genetic testing should increase, given their primary role in the preservation of genetic resources of one of the most important pollinators worldwide.



Genetic Diversity and Population Structure of Spanish Goat Breeds

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POSTER SESSION 2 - P02.068, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

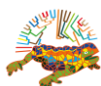
Background: Goats were domesticated around 10,000 years before present (YBP) in Southwest Asia and reached the Iberian Peninsula around 7000 YBP. Historically, goats were used to produce meat and milk and nowadays 22 caprine breeds have been officially recognized in Spain, of which 16 have a status of special protection to ensure their conservation. The main goal of the current work was to characterize the genetic diversity of Spanish goat breeds as well as to elucidate their population structure and genetic relationships using high throughput genotyping data.

Materials and methods: We collected either blood samples, DNA samples or Goat SNP50 BeadChip data from 19 Spanish goat breeds including Azpi Gorri, Blanca Andaluza, Blanca Celtibérica, Blanca de Rasquera, Bermeya, Cabra de las Mesetas, Cabra del Guadarrama, Eivissenca, Murciano-Granadina, Malagueña, Florida, Mallorquina, Moncaína, Negra Serrana, Palmera, Payoya, Pirenaica, Retinta, and Verata. Whenever required, genomic DNA was extracted from blood, and genotyping was performed with the Goat_IGGC_HTS_v3 BeadChip from Illumina which encompasses 79,538 single nucleotide polymorphism (SNP) markers. The PLINK software was used to filter out individuals and SNP markers. The final dataset included 457 Spanish goat samples and 41,626 variants. We also estimated the genomic inbreeding coefficient (FROH) as the proportion of runs of homozygosity (ROH) over the total length of autosomes using the R package “detectRun”.

Result: The Palmera breed had the lowest values of observed ($H_o = 0.288$) and expected ($H_e = 0.284$) heterozygosities, followed by the Balearic breeds Eivissenca ($H_o = 0.368$, $H_e = 0.382$) and Mallorquina ($H_o = 0.370$, $H_e = 0.377$). Performance of principal component analysis (PCA) of Spanish breeds revealed

one single cluster containing most Spanish breeds, that were weakly differentiated at the genetic level, with the only exception of Cabra del Guadarrama, that had a scattered distribution, and Palmera. This latter finding was anticipated because the Palmera breed probably originated from goats that were transported by Imazighen peoples to the Canary Islands approximately 2000 YBP. A second PCA excluding Palmera and Guadarrama evidenced a high differentiation of two Balearic breeds, Mallorquina and Eivissenca, and the Retinta breed that is mainly distributed in Southwest Spain. This result might be due to the insular origin of these two breeds as well as to their reduced population size, two factors that might have enhanced the impact of genetic drift on allele frequencies. We have also observed a south to north axis of diversity in the main cluster of Spanish breeds, with Northern Spanish breeds (Moncaína, Bermeya and Pirenaica) in one extreme and Southern Andalusian breeds (Murciano-Granadina, Malaguená and Florida) in the other one. Consistent results were obtained when analyzing genetic data with ADMIXTURE ($K=12$) The highest levels of inbreeding were found in Moncaína ($FROH = 0.096$) and Mallorquina ($FROH = 0.093$) breeds, likely due to their small population sizes. Indeed, the Moncaína breed was in danger of extinction at the beginning of the 21st century, with barely three hundred individuals.

Conclusions: Spanish goat breeds show high levels of genetic diversity and low FROH except for a few breeds that have undergone strong founder effects or population bottlenecks. The most differentiated breeds are Palmera, Guadarrama, Mallorquina, Eivissenca, Payoya and Blanca de Rasquera, while the remaining Spanish breeds are weakly differentiated.



Genetic surveillance reveals low, sustained malaria transmission with clonal replacement in Sao Tome and Principe

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POSTER SESSION 2 - P02.070, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Despite efforts to eliminate malaria in Sao Tome and Principe (STP), cases have recently increased. Understanding residual transmission structure is crucial for developing effective elimination strategies. This study collected surveillance data and generated amplicon sequencing data from 980 samples between 2010 and 2016 to examine the genetic structure of the parasite population. The mean multiplicity of infection (MOI) was 1.3, with 11% polyclonal infections, indicating low transmission intensity. Temporal trends of these genetic metrics did not align with incidence rates, suggesting that changes in genetic metrics may not straightforwardly reflect changes in transmission intensity, particularly in low transmission settings where genetic drift and

importation have a substantial impact. While 88% of samples were genetically linked, continuous turnover in genetic clusters and changes in drug-resistance haplotypes were observed. Principal component analysis revealed some STP samples were genetically similar to those from Central and West Africa, indicating possible importation. These findings highlight the need to prioritize several interventions such as targeted interventions against transmission hotspots, reactive case detection, and strategies to reduce the introduction of new parasites into this island nation as it approaches elimination. This study also serves as a case study for implementing genetic surveillance in a low transmission setting.



Genome Scans of Adaptive Introgression in the genomically eroded Iberian Lynx (*Lynx pardinus*)

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POSTER SESSION 2 - **P02.099**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

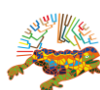
Background: The Iberian lynx (*Lynx pardinus*) experienced a severe population collapse, reaching fewer than 100 individuals and placing it at the edge of extinction by 2002. Its demographic history, marked by low effective population sizes and serial bottlenecks, have resulted in exceptionally low levels of genomic diversity—among the lowest reported in mammals. Despite evidence for an overall loss of genetic diversity during the last decades, ancient DNA analyses revealed an increase in diversity in the last 4.000 years caused by introgression from the Eurasian lynx (*Lynx lynx*). Indeed, paleontological and genomic data supports extensive and recurrent, gene flow between both species, allowed by periods of range overlap in the Mediterranean basin since late Pleistocene and throughout Holocene. The fitness consequences of introgression remain unexplored, but an intriguing possibility is that introgression has restored functional diversity, resulting in adaptive introgression.

Material and methods: In this study, we perform genome-wide scans for adaptive introgression in *L. pardinus* using VolcanoFinder and high-coverage whole-genome sequencing data from 15 to 45 individuals. The top 1 % of VolcanoFinder outliers are taken as candidate regions and intersected with a newly developed *L. pardinus* genome annotation to identify

genes and gene functions potentially involved in ecologically relevant adaptations. Signals located within centromeric and telomeric regions have been excluded in order to reduce false positives. We then perform an over-representation analysis (ORA) on these gene sets to detect significantly enriched functional categories ($p < 0.05$). Finally, we assess the overlap between our candidate regions and those showing evidence of introgression (Bazzicalupo et al., in prep.) or positive selection (Lorenzo-Fernández et al., in prep.).

Results: This analysis revealed eight candidate regions of adaptive introgression distributed across five chromosomes. Within these regions, we identified a total of 337 genes and 1,748 associated Gene Ontology (GO) terms. The functional categories include immune and inflammatory response, tissue development and cell differentiation, lipid energy metabolism and neural functions.

Conclusions: This integrative analysis will contribute to a deeper understanding of the fitness consequences of introgression and the evolutionary mechanisms that have sustained adaptive evolution of *L. pardinus* despite its reduced genetic diversity.



Genomic consequences of recent population decline in the endangered butterfly *Hipparchia sbordonii*

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POSTER SESSION 2 - **P02.104**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: *Hipparchia sbordonii* is an endangered butterfly endemic to the Pontine Islands, Tyrrhenian Sea, Italy. Understanding the genomic consequences of small and isolated populations is key to conservation genomics. We investigated genome-wide patterns of genetic diversity, genetic load, and demographic history in *H. sbordonii*, integrating data from historical (1990) and recent (2019) samples. As a comparative framework, we included the widespread congener *H. semele*, characterized by larger and more continuous populations.

Materials and Methods: Using whole-genome resequencing data from 28 individuals— ten *H. sbordonii* from Ponza (2019), three historical *H. sbordonii* from Ponza and three from Palmarola (1990), ten *H. semele* from the mainland (2019), and two outgroup species (*H. fagi* and *H. mersina*) — and a high-quality reference genome for *H. sbordonii*, we performed recent demographic reconstructions using GONE, estimated population genetic diversity (π , Tajima's *D*), inferred runs of homozygosity (ROH), and examined the functional impact of variants using SNPeff. This study is part of the ENDEMIXIT project (www.endemixit.com), which focuses on the population genomics of Italian endemic species.

Results: Genetic diversity (π) was substantially lower in *H. sbordonii* (mean $\pi = 3.579 \times 10^{-3}$) than in *H. semele* (mean $\pi = 6.525 \times 10^{-3}$), consistent with expectations for small populations. Demographic reconstructions based on recent samples revealed a marked decline in effective population size in *H. sbordonii* over the last

~30 generations, likely reflecting recent demographic contraction.

Tajima's *D* was near zero in historical samples of *H. sbordonii*, but showed a marked increase in recent samples, suggesting a signal of recent demographic contraction. In contrast, *H. semele* exhibited strongly negative Tajima's *D* values, consistent with population expansion.

To quantify genetic load, we used two outgroup species (*H. fagi* and *H. mersina*) to polarize derived alleles and calculated both masked and realized load per individual. Results revealed higher realized load in *H. sbordonii* compared with *H. semele*, consistent with reduced effective population size and increased inbreeding, as supported by a higher abundance and length of runs of homozygosity (ROH). Conversely, *H. semele* exhibited higher masked load, in line with its larger effective population size.

Interestingly, relative derived allele frequency comparisons (R'_{xy}) between *H. sbordonii* and *H. semele* showed a depletion of high-effect mutations in *H. sbordonii* (R'_{xy} High effect < 1), suggesting possible purging. However, moderate- and low-effect mutations were enriched (R'_{xy} Moderate effect > 1 , R'_{xy} Low effect > 1), potentially reflecting island-specific adaptations under relaxed purifying selection or directional selection in the insular environment.

Conclusions: Our findings illustrate the genomic consequences of recent decline and short-term small population size in *H. sbordonii*, with implications for its conservation. Ongoing habitat loss could exacerbate genetic erosion. Future work will include additional historical samples.



Genomic Insights into Anthropogenic Impacts on Small Mammal Populations Since the Natufian Period

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*POSTER SESSION 2 - **P02.065**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM*

At the intersection of three continents, one of the world's most ecologically and culturally dynamic regions has served as a critical corridor for both human migration and faunal exchange since prehistoric times. This biodiversity hotspot, rich in endemic species and archaeological heritage, offers an opportunity to investigate how environmental and anthropogenic forces shape genomic diversity across millennia. Focusing on the Hula Valley ecosystem in Israel - once a vast wetland and now a landscape transformed by millennia of cultivation, drainage, and partial restoration (old times rewilding) we explore how repeated human interventions have affected the evolutionary trajectories of native mammal populations. Our study centers on small, vulnerable populations of the mountain gazelle (*Gazella gazella*), jungle cat (*Felis chaus*), and wildcat (*Felis silvestris*). We integrate genomic data from modern populations with historical samples (ca. 1960s) and ancient remains from the

Natufian period (~13,000 BP), including specimens from the Eynan (Ain Mallaha) archaeological site. Applying robust ancient DNA (aDNA) methods tailored to humid environments—from well-known protocols for aDNA extraction, in-house and commercial kits for dsDNA Library Prep for Illumina—we successfully recovered reads from genome-wide data from the degraded materials. Analyses of genetic diversity, demographic history, and population structure reveal pronounced temporal shifts linked to natural environmental changes and human land-use practices. These findings underscore the value of museomics and long-term population genomics in understanding how small populations adapt—or fail to adapt—under persistent anthropogenic pressure. Our work provides a critical evolutionary context for conservation planning and highlights the importance of integrating ancient and modern data to inform biodiversity resilience in an era of rapid ecological change.



Genomic Insights into the Evolution of Inbred Loach Populations: Phylogeny and Runs of Homozygosity

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POSTER SESSION 2 - **P02.074**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

In nature, small, isolated populations often develop unique genetic characteristics. Such populations also experience reduced genetic diversity and increased homozygosity due to restricted gene flow and genetic drift, elevating their extinction risk and limiting their adaptive potential. Understanding the evolutionary dynamics of small, isolated populations is thus critical not only for unraveling the genetic consequences of demographic processes such as bottlenecks, inbreeding, and genetic drift, but also for addressing conservation strategies. While these patterns and processes have been extensively studied in laboratory populations and a handful of populations of iconic mammalian species, highly replicated studies of wild vertebrates remain rare. Therefore, the flat-headed loach (*Oreonectes platycephalus*), a small freshwater fish occurring in highly fragmented habitat, offers a valuable opportunity to study genetic consequences of isolation and small population size in highly replicated fashion.

Analysis of genome wide genetic diversity across 31 loach populations from southern

China was performed with the aid of whole genome re-sequencing data. Runs of Homozygosity (ROH) analysis indicated a predominance of short ROH segments (<100 kbp) in all populations, suggesting historical inbreeding. Inbreeding coefficients estimated from ROH (FROH) were particularly high in Hong Kong populations, averaging 0.37 and ranging dramatically from 0.11 to 0.72. Further analysis of ROH characteristics (total length and count) suggested that many of the populations have experienced population size bottlenecks and are consanguineous. To better understand their demographic history and evolutionary potential, we are now estimating their contemporary effective population sizes (N_e) and deleterious mutation loads, as well as evaluating to what degree the variation in these parameters are explainable by shared phylogenetic and demographic history. Our results so far suggest that many of the studied populations are likely to have severely reduced evolutionary potential and may suffer from increased load of deleterious mutations.



Genomics of caribou (*Rangifer tarandus*) in Western Canada: an assessment of inbreeding and demographic history

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POSTER SESSION 2 - P02.085, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Conservation genomics plays a critical role in preventing population extinction by providing insights into genetic health and population dynamics. Inbreeding, a common consequence of small populations, reduces genetic diversity and increases extinction risks. Additionally, genetic drift further reduces effective population size (N_e) in small or isolated populations, exacerbating the loss of diversity. N_e is increasingly recognized as a key indicator for assessing genetic variation and the effects of genetic drift, particularly in vulnerable populations. In Western Canada, many caribou subpopulations are in decline or endangered, making the preservation of genetic diversity a priority under the federal Canadian Species at Risk Act (SARA). Increased predation and habitat degradation have led to smaller census sizes (N_c) and reduced connectivity between subpopulations, which limits gene flow. Specifically, several subpopulations have low census sizes, with some considered functionally extirpated and in decline of British Columbia. To identify at-risk subpopulations, we applied genomics tools to assess key indicators of genetic health. Using approximately 32,000 Single Nucleotide Polymorphisms (SNPs), we estimated inbreeding and contemporary and historical N_e for 45 subpopulations ($n = 759$) and 6 metapopulations across Western Canada. Inbreeding was assessed using Runs of Homozygosity (ROHs), and

N_e was estimated using LD-based methods implemented in the software 'currentNe' and 'GONE'. Our results show that smaller, more isolated subpopulations exhibit higher proportions of ROHs, indicating greater inbreeding. Shorter ROHs (<2 Mb) suggest historical inbreeding in all subpopulations, while longer ROHs (>8 Mb) are more common in smaller, endangered subpopulations. Notably, some subpopulations that were not previously known to be endangered showed high levels of inbreeding. Most subpopulations had low N_e estimates, with many falling below the critical threshold of 50, raising concerns for their short-term survival. Additionally, many subpopulations and all metapopulations had N_e estimates below 500, signaling risks for long-term viability. Contemporary N_e was generally smaller than N_c , and smaller subpopulations exhibited a higher N_e/N_c ratio. Historical trends in N_e show significant genetic bottlenecks in northern subpopulations between 1750–1850 and southern ones between 1900–1950, likely driven by climate change (end of Little Ice Age) and human activities before and after Euro-American settlement in Western Canada. Our study provides important insights into the genetic health and demographic trends of caribou in Western Canada, offering key indicators for active management, identifying sub populations 'at-risk', and implementing both short- and long-term conservation strategies.



Habitat-Driven Divergence: Early Development Variation in Two Ecotypes of the Marine Gastropod *Littorina fabalis*

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POSTER SESSION 2 - **P02.103**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Small populations often face strong selective pressures, particularly in fragmented or ecologically contrasting habitats. The marine gastropod *Littorina fabalis* offers a powerful model for studying evolutionary divergence in such environments, as it comprises two ecotypes adapted to distinct microhabitats: a large-shelled form inhabiting moderately wave-exposed rocky shores, and a dwarf form restricted to sheltered areas. This species is distributed across the northeast Atlantic, and the present research was conducted on the Swedish west coast, where both ecotypes coexist among brown macroalgae, and the described ecotypes can be found. In this region, the ecotypes occasionally hybridize in contact zones, yet maintain striking phenotypic and genetic divergence. Genetic differentiation is particularly pronounced within 12 large, linked chromosomal inversions, which act as strong barriers to gene flow. While the ecotypes' differences in adult morphology and habitat preference are well documented, the potential role of early developmental traits in driving or reinforcing this divergence remains poorly understood.

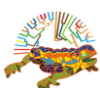
In this study, we investigated whether microhabitat conditions shape early developmental traits in *L. fabalis* and whether these traits differ between ecotypes. We focused on the vulnerable phase from gastrulation to veliger stage, hypothesizing that each ecotype would perform better in its native habitat. We conducted two complementary experiments: a controlled temperature treatment in the laboratory (5°C, 10°C, 15°C, 20°C, 25°C) and a reciprocal transplant experiment between two field sites representing sheltered and exposed habitats. Egg masses from both ecotypes were collected and development time was tracked under stereo microscopy. Environmental parameters (temperature, salinity, oxygen) were

logged at each site to contextualize developmental differences.

Results showed that temperature had a clear effect on development: while little variation was seen between 10°C and 15°C, at 20°C the dwarf ecotype exhibited faster development compared to the large ecotype. However, in the field transplant experiment, the dwarf ecotype developed the slowest in both habitats. The large ecotype developed faster overall, and performed particularly well in its native exposed site, suggesting possible local adaptation. These findings highlight the complexity of environmental interactions in shaping life-history traits, particularly in small, partially isolated populations.

Both ecotypes showed a preference for laying egg masses on *Fucus vesiculosus*, regardless of habitat, suggesting that substrate availability or surface properties might also influence reproductive behavior. We also observed notable variation in egg mass morphology between ecotypes, as well as differences in microalgal colonization, particularly in the dwarf ecotype. These observations raise new questions about the potential functional significance of egg mass characteristics in buffering against microhabitat stressors such as temperature fluctuations or desiccation.

We are currently developing a developmental staging map for *L. fabalis* embryos and plan to combine proteomic analyses of egg masses with extended environmental monitoring. By integrating developmental, ecological, and molecular approaches, we aim to better understand how early life stages contribute to ecotype divergence in marine gastropods. Our work highlights how fine-scale environmental heterogeneity can drive evolutionary divergence, even in small coastal populations with limited dispersal.



Landscape driven isolation and inbreeding in specialist grassland butterflies

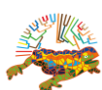
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*POSTER SESSION 2 - **P02.107**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM*

Land use change from agricultural intensification has been highlighted as one of the major drivers of global declines in insects, reducing species diversity and abundance and shifting community composition towards generalist species. However, it remains a challenge to determine how land use change impacts the genetic diversity of species and populations even if this diversity is vital to their long-term persistence. Reductions in genetic diversity are expected to reduce adaptive potential and increase the frequency of deleterious mutations, potentially reinforcing demographic declines. We investigated to what extent grassland habitat loss associated with agricultural intensification in southern Sweden has resulted in genetic diversity declines in grassland butterflies from genomic data across multiple species to infer if differential demographic decline in generalists and specialists is mirrored by patterns of genetic erosion. Through a spatial comparison of populations of three grassland butterfly species, we uncover that specialist species show stronger genetic structure, with one specialist species, *Cyaniris semiargus*, experiencing sufficient isolation to lead to higher rates of recent inbreeding compared to the other species. Associating genetic diversity with land cover variables, we find that genetic diversity is lower in populations with less grassland area in the surrounding landscape due to higher rates of inbreeding. Comparisons between

genomes from contemporary and museum specimens of these same three species reveal the population isolation in this specialist species has arisen over the past century, increasing rates of inbreeding and reducing genetic diversity. Importantly, inbreeding has increased homozygosity of putative weakly deleterious mutations in this species, which may depress fitness of inbred individuals and reinforce decline. To determine if these patterns are general, we estimated the extent of genetic differentiation, reductions in effective population size, and recent inbreeding in Sweden's most common grassland butterflies and the European Grassland Butterfly Indicator species. While estimates of heterozygosity varied by species, rates of inbreeding were distinctly higher in specialists, as were levels of genetic differentiation between localities. Trajectories of contemporary effective population size over time reveal that while many widespread species maintain high effective population sizes, specialists have dropped to sizes below 500 and even 50 over the past 30-50 years. Together, these findings highlight that land use change has reduced functional connectivity and increased inbreeding in specialist grassland butterflies, and conservation efforts targeting habitat restorations that restore gene flow are necessary for the persistence of specialist grassland insect species.



Long-term genomic and morphological evolution of island vertebrates

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POSTER SESSION 2 - P02.102, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Biodiversity can be defined across three hierarchical levels: ecosystem diversity, species diversity and genetic diversity. Among these, endemic species from small, isolated populations are of particular interest due to their unique evolutionary trajectories and increased vulnerability to environmental challenges. Islands, as biodiversity hot-spots, are characterized by a high number of endemic species and serve as natural laboratories for studying evolutionary processes in fragmented environments. Understanding the long-term dynamics of these populations is essential to assess species' adaptive capacities and resilience and to inform conservation biology managerial tactics. Technological advances now allow the study of morphological and genetic diversity in ancient populations, enabling the reconstruction of past demographic histories, the detection of genetic erosion, and the identification of early anthropogenic impacts. By integrating present-day genomics, paleogenomics and

morphological analyses, we aim to build a comprehensive view of how small populations respond to environmental pressures over time. In this work, we present an integrative study of two insular endemic vertebrates from the Balearic Islands: the reptile *Podarcis lilfordi* and the amphibian *Alytes muletensis*. Both species are characterized by small fragmented populations highly susceptible to genetic drift, inbreeding and environmental stress. By comparing ancient and modern mitochondrial genomes and morphological traits, we aim to quantify genetic erosion, assess microevolutionary changes linked to demographic fluctuations and human colonization events. Our approach provides a temporal perspective on the evolutionary dynamics of small, isolated populations, offering insights relevant for understanding resilience and vulnerability of insular endemic species in the face of ongoing anthropogenic pressures.



Loss of genetic variation and establishment of damaging variants in effectively small populations

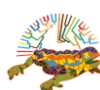
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*POSTER SESSION 2 - **P02.081**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM*

Domestic animal populations, as well as many animal populations in the wild, are effectively small and shrinking. In particular, in domestic animals with breeding programs, there is very strong directional selection, while breeds without modern breeding tend to be fragmented and in decline. Large-effect deleterious variants at relatively high frequencies are common enough in domestic animals that they pose applied problems for breeders. In our work, we have combined genomic data analysis with a

series of population genetic models to address the loss of genetic variation in effectively small and shrinking populations, and how large-effect deleterious variants establish themselves in small populations. The results suggest the presence of transient genetic variation, predicted to be gradually lost, and that balancing selection by heterozygote advantage, even transiently through close linkage, makes establishment of new large-effect deleterious alleles likely.



Mapping extinction risk in seahorses: integrating genomics, phylogeny, ecology and policy towards evolution-guided conservation

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POSTER SESSION 2 - **P02.069**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Seahorses (*Hippocampus* spp.) are flagship animals with unique adaptations that inspire numerous conservation programs. They are the first marine genus to be fully listed on the Convention on International Trade in Endangered Species (CITES) Appendix II due to their substantial demographic decline, vulnerability to overexploitation, and habitat loss. The peculiar life history of these fishes has been widely addressed through evolutionary and ecological analyses. Yet, no study has integrated current knowledge to approach species-based conservation status, including trends in abundance, diversity, and threats, hindering seahorses' worldwide effective assessment and management.

Materials and methods: We bridge these gaps by taking advantage of the available geographic, ecological, and genomic (ultra-conserved elements and whole genomes) data in the *Hippocampus* species at a global scale and present the most comprehensive study of the seahorses' conservation status to date. We explore seahorses' diversity patterns in space and time by using species distribution modelling, phylogenomic mapping, comparative genomic applications, and conservation gap analyses. We consider their evolutionary history and genomic erosion, including genetic load, which have been largely disregarded by current conservation policies. We further assess the degree and type of protective measures currently

granted to seahorses and the biological and ecological factors that are contributing to their existing and future extinction risk.

Results: We showed that, despite being charismatic animals, their current level of protection is poor, particularly concerning their evolutionary diversity. We highlighted areas and species to be prioritized for urgent conservation interventions. Although genomic erosion appeared not to be an immediate concern in these species, most seahorses revealed a decline in their genome-wide diversity and an increased masked genetic load that may reduce their viability in the future. Our results raise questions on whether the current conservation indicators and practices are effective in preserving the present diversity and function of these iconic fishes, as well as their future evolutionary potential and ecological resilience.

Conclusions: These insights provide a unified, interdisciplinary perspective on seahorse conservation, emphasizing the need to integrate evolutionary history and genomic vulnerability into management policies. As "omics" and open-access data expand, we highlight unprecedented opportunities for research-driven conservation—ensuring these iconic fishes persist in the Anthropocene. Our framework serves as a model for assessing and protecting taxa of conservation concern worldwide.



Microevolutionary changes of *Daphnia galeata* during the oligotrophication of Lake Constance

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POSTER SESSION 2 - **P02.082**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

This study investigates how *D. galeata* has adapted to environmental changes associated with the oligotrophication of Lake Constance, a deep, peralpine lake in Central Europe. Using a resurrection ecology approach, we established *Daphnia* clones from *Daphnia* resting eggs deposited in various sediment depths representing time periods with high nutrient concentrations (eutrophic: 1970 and 1980s) vs. low nutrient concentrations (oligotrophic: 1995 - 2015). We subsequently used two common-garden experiments to compare the performance of clones from the two time periods to a) the presence of predator kairomones and b) food availability.

Our predator kairomone experiment revealed that *Daphnia* from oligotrophic clones developed longer spines, but smaller heads and eyes regardless of predator presence, indicating microevolution of these body traits, but no evidence for evolution of phenotypic plasticity. The food availability experiment showed that under low food conditions, all clones plastically adjusted

their filter combs by increasing their size and setae length, but we found no differences between clones from the eutrophic versus the oligotrophic period. In contrast, the response of *Daphnia* life-histories traits differed between periods suggesting evolution of plasticity: *Daphnia* from the oligotrophic period showed later reproductive timings at low food concentrations compared to eutrophic clones.

These findings suggest that *D. galeata* showed a complex response to environmental changes associated with the oligotrophication of Lake Constance. This response included phenotypic plasticity, microevolution as well as evolution of plasticity of traits associated with food uptake, predator defense, and life history adjustments. We thus provide insights into how organisms integrate evolutionary and plastic responses to cope with environmental changes which likely will shift the relative importance of bottom-up versus top-down control for fitness.



Mitochondrial Divergence and Population Genomics in a Dwarf Insular Snake

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POSTER SESSION 2 - **P02.073**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Island ecosystems often host organisms distinct from their mainland counterparts in physiology, behavior, and morphology. A particular trend observed in both extinct and extant organisms on islands is a drastic increase or reduction in body size. The Channel Islands, off the coast of California, are home to several endemic species exhibiting reduced body sizes relative to the mainland; one of these is the Channel Islands gopher snake, *Pituophis catenifer pumulis*. We have been collecting morphological, physiological, and genetic data from two of the Channel Islands (Santa Cruz (SCI) and Santa Rosa (SRI)) along with northern (MN) and southern mainland (MS) California locations, and have demonstrated that island individuals are 30% smaller than their mainland counterparts, *Pituophis catenifer subsp.* The islands have experienced environmental degradation from human activity since the 19th century, but little is understood about the impact on endemic reptiles. These populations may experience dissimilar evolutionary forces compared to their mainland counterparts, with genetic drift likely playing a strong role in shaping diversity in neutral and functional genetic variation. Serpentes exhibit some of the highest mitochondrial substitution rates among vertebrates, and island populations across taxa frequently display significantly elevated non-synonymous to synonymous substitution rate ratios. This makes the mitochondrial genome particularly important for understanding the potential effects of genetic drift on functional divergence in isolated populations. By contrasting island and mainland populations, we can evaluate genetic diversity metrics, divergence between subspecies, and identify potentially functional mutations. Using whole genome sequencing data from 77 *P. catenifer* individuals across islands and mainland locations, we analyze demography at the level of

single-nucleotide polymorphisms (SNPs) from mitochondrial genomes. We found both islands contain their own unique mitochondrial haplogroups, with SCI containing two haplogroups and SRI containing one haplogroup. Haplotype maps indicate that at the mitochondrial level, island populations are more closely related to the southern mainland, which concurs with literature focused on only one mtDNA region. Pairwise genetic distances also show that SCI and MS are most closely related ($F_{st}=0.10$), and MS and MN are most distantly related ($F_{st}=0.89$). Interestingly, SCI exhibits higher nucleotide diversity compared to the mainland (SCI $\pi = 0.0049$, MS $\pi = 0.0016$). We also discovered several amino acid changes found in high frequencies on the islands relative to the mainland, a few that were private to one of the islands. On SCI, three of these five amino acid changes are clustered on NADH genes in Complex I and are found on the same haplotype. On SRI, four of these six amino acid changes are clustered on COX genes in Complex IV on the same haplotype. Whether due to selection or mutation accumulation due to drift, the non-random distribution of the amino acid changing haplotypes suggests the potential for functional consequences to mitochondrial performance that is being evaluated further. Presented results will include an expanded sample size that brings insight into a third island and a third mainland location, along with predictions of if island-unique mutations are functional. This research provides novel information about the population status and evolutionary history of *P. catenifer*, contributing to a broader understanding of how insular environments shape genomic divergence. Our data lays the groundwork for downstream functional genomics analyses to understand the genetic basis for the morphological and physiological differences between mainland and island populations.



Mutation bias influences adaptive evolution, even at large population sizes.

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POSTER SESSION 2 - **P02.055**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Introduction: Genetic mutation has long been seen as solely a stochastic force in adaptation. However, organisms can present with mutational biases, influencing which mutations arise at which positions, and evidence is showing an increasing role in dictating evolutionary pathways. Current consensus is that mutation biases can only play a prominent role in evolution when population sizes are small, and the power of selection is reduced. Understanding the scenarios in which mutation bias will play a critical role is necessary for predicting adaptive outcomes in natural environments and clinical settings.

Objective: To understand the role of population size in the predictive outcomes of mutator strain evolution to strong selection pressures.

Method: To address this, we evolve two *Escherichia coli* strains with distinct mutator alleles to doubling concentrations of the antibiotic cefotaxime, over a range of effective population sizes, and profiled the resistance alleles that fixed. Crucially, both

strains contain the beta-lactamase TEM-1, and only one mutator elevates first step mutations that increase cefotaximase activity.

Findings: We found a difference in survivability between the mutator strains, as well as increased survivability at larger population sizes. Furthermore, we found that whilst larger populations were able to find mutations associated with TEM-1, all populations still fixed resistance alleles associated with their mutation bias. Smaller sized populations showed mutational bias playing a dominant role, but interestingly each mutator strain responded to declining population sizes differently. We found extensive differences in resistances to other antibiotics as well, having implications for antibiotic combination treatments in clinical

Conclusion: This work shows that mutational biases can play an outsized role in evolution, and must be integrated into current evolutionary thinking in order to be able to anticipate evolutionary trajectories in adapting populations.



On the edge of extinction: genetic monitoring of the Aeolian wall lizard in a fragmented island system

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POSTER SESSION 2 - P02.108, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The Critically Endangered Aeolian wall lizard (*Podarcis raffonei*) is an island endemic of the Aeolian Archipelago in Southern Italy. The species is currently recorded in only four locations: three tiny islets and the small peninsula of Capo Grosso on the island of Vulcano. With an estimated range of ~1.6 ha, a severely fragmented distribution, and a continuing decline in the number of mature individuals, urgent actions are necessary to monitor the genetic viability and conservation of the Aeolian wall lizard. An additional threat is competition and hypothesised widespread hybridisation with the introduced Italian wall lizard (*P. siculus*). In particular, the largest population of *P. raffonei* survives on a narrow peninsula (Capo Grosso) that is mildly connected to the island of Vulcano, which has been entirely invaded by *P. siculus*. Recent observations of individuals with intermediate phenotypes raised significant concern that hybridisation could swamp this last genetic stronghold. We genetically characterised lizards from Capo Grosso - including individuals with native, invasive, and intermediate phenotypes - and found a low hybridisation rate (~3%), with only

two F1 hybrids and two backcrosses. These results suggest that hybridisation is not currently a major concern in Capo Grosso. However, the population exhibits low genetic diversity, a small effective population size, and a low N_e/N_c ratio, while competition with *P. siculus* continues to pose a serious challenge to the species' persistence. As management strategies are urgently needed to control *P. siculus* and preserve the genetic diversity of *P. raffonei*, we initiated the LIFE EOLIZARD project, which includes a captive breeding program to address the species' decline and risk of extinction. We are undertaking a reintroduction of captive-bred *P. raffonei* individuals to two new islets, with the goal of establishing a sanctuary that will support the species' long-term conservation and serve as a safeguard for the species' genetic viability. To inform these efforts, we have performed a genomic screening for the selection of breeders to assess: 1) potential intraspecific hybrids; 2) genetic relatedness; 3) sex; and 4) genetic viability. Our future work will harness genetic monitoring methods to track the fitness of all introduced Aeolian wall lizards.



Patterns of inbreeding and purging selection in sand lizard populations in Sweden

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POSTER SESSION 2 - **P02.100**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Sand lizards (*Lacerta agilis*) in Sweden have experienced a steep decline in the last decades due to loss of suitable habitat and fragmentation of originally connected populations. As the populations get smaller, there is increasing concern about inbreeding depression. Local extinctions have been widely documented especially in the northern range, where the populations are highly isolated; however, several populations have also shown a remarkable recovery following habitat restoration.

Using whole-genome data from 11 eleven locations covering the entire range of distribution, we determine the inbreeding status of the populations by analysing runs of homozygosity, as their length can be informative in dating the occurrence of consanguineous mating. Then we test the hypothesis that highly isolated small populations have a lower mutation load compared to larger more connected

populations. A stronger effect of purging selection in small, inbred populations has been reported in several other species but has rarely been studied in reptiles. We will verify whether the combination of increased inbreeding, which brings recessive variants in homozygosity, and purifying selection, which purges harmful mutations from the gene pool, is able to slow down the accumulation of deleterious variation.

Preliminary results confirm overall low levels of heterozygosity, with extremely high inbreeding coefficients in the northern range. This research, coupled with spatial analyses and forward simulations, will provide key insights for further work aimed at the conservation of this endangered species, for example, to identify: i) populations in critical need of management measures, ii) suitable populations for translocations, iii) effective strategies to improve connectivity.



Pleistocene climate and human impact shaped the demographic history of Mediterranean gray wolves (*Canis lupus*)

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POSTER SESSION 2 - **P02.083**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Climate change and human activity are among the most significant drivers of biodiversity loss, contributing to dramatic declines and local extinctions of wild species. Apex predators are particularly vulnerable due to their high energy requirements, small effective population sizes, and frequent targeting by humans—whether as hunting trophies or perceived threats. One of the most persecuted apex predators is the gray wolf (*Canis lupus*), a Palearctic species historically widespread across Eurasia and North America and well represented in the European fossil record since the Pleistocene. During the last centuries and due to human hunting, many gray wolf populations in Western and Southern Europe have undergone drastic declines, with widespread local extinctions and severe bottlenecks, particularly during the 20th century. In this study, we investigate how Pleistocene climate fluctuations and anthropogenic pressures shaped the

demographic history of gray wolves across the Mediterranean. We analyzed genomic data from 72 individuals representing three Mediterranean populations and inferred demographic changes using Markovian Coalescent-, Site Frequency Spectrum- and Linkage Disequilibrium-based approaches. Our results reveal a correlation between Pleistocene climatic events and fluctuations in effective population size. Additionally, we detect a negative association between wolf population size and human presence in several regions across the Holocene. Finally, we discovered strong genetic bottlenecks associated to systematic extermination campaigns carried out during the 20th century across multiple Mediterranean countries. Our findings highlight the long-term vulnerability of apex predators to both environmental and anthropogenic pressures and emphasize the importance of integrating genomic data and historical context in conservation strategies.



Population Genomics of Laboratory Zebrafish: Insights into Domestication and genetic diversity

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POSTER SESSION 2 - P02.059, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Zebrafish (*Danio rerio*) are a central model in biomedical and developmental research, yet the population structure and domestication history of laboratory strains remain underexplored in an evolutionary context. Here, we present a comparative genomic analysis of five zebrafish strains—three sequenced in this study (TU, AB, JU) and two from public repositories (T5D, MA)—to investigate the genomic impact of domestication and inbreeding, with a particular focus on genetic diversity, population structure, and genes encoding epigenetic regulators.

Using shallow whole-genome resequencing of 95 individuals, we uncover substantial variation in genetic diversity between strains. Common laboratory strains such as TU and AB show extensive runs of homozygosity, consistent with inbreeding and genetic

bottlenecks, whereas wild-derived JU and MA retain higher heterozygosity. Demographic reconstruction via SMC++ suggests historical selective sweeps in all strains dating back ~1000 years, possibly linked to regional climatic shifts. Notably, genes under selection differ markedly between wild and domesticated strains, with a subset of loci consistently identified across domesticated lines, suggesting shared signatures of laboratory adaptation.

Our study enhances the resolution of zebrafish population genomics and provides essential context for future work in adaptation, selection, and domestication. The expanded individual-based genomic resource will serve the zebrafish community working at the interface of genetics and evolution.



Population genomics of the genus *Megasyllis* (Annelida, Syllidae) reveals hidden connectivity in the Canary Islands

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POSTER SESSION 2 - **P02.089**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Syllid polychaetes are a group of marine annelids, inhabiting a wide range of oceanic environments worldwide. Despite their abundance and ecological importance, the population structure and genetic connectivity within the species of Syllidae family remains largely unexplored. In this study, we used a combination of genome-wide SNP data generated by RAD-seq and mitochondrial markers from multiple populations of a new species of *Megasyllis* sampled in 12 localities on four islands of the Canary archipelago (western Atlantic, Spain). Although the species was quite common across the different sampling sites in La Gomera and Tenerife islands, any specimen of *Megasyllis* sp. nov. was found on the easternmost islands of Fuerteventura and Lanzarote. Preliminary genetic analyses

in 83 individuals indicate high levels of gene flow among populations of the two sampled islands, suggesting a substantial connectivity of the studied species in this area. Moreover, we provide the first evidence of amphi-Atlantic genetic connectivity within the family Syllidae, with shared haplotypes detected among populations from the Canary Islands and Bermuda archipelago. These findings represent a novel biogeographic pattern for syllid polychaetes and underscore the potential role of long-distance dispersal mechanisms in shaping their population dynamics. Our results contribute to a more comprehensive understanding of the evolutionary processes influencing genetic diversity and connectivity in marine benthic invertebrates.



Purging and Accumulation of Genetic Load in an Expanding Population of Trinidadian Guppies

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POSTER SESSION 2 - **P02.076**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Genetic load refers to the presence of deleterious alleles in a genome that result in reduced fitness, often through homozygous genotypes. Demographic history can shape the genetic load of a population by influencing the efficacy of selection and levels of heterozygosity. Understanding these processes is essential for identifying threats to population viability and predicting evolutionary trajectories, especially in bottlenecked populations, such as those translocated by humans into non-native environments. These founder events may impact genetic load and shape a population's invasive potential. Here, we examine the causes and consequences of expansion in Trinidadian guppies (*Poecilia reticulata*) by estimating genetic load across the Turure River population, where translocated individuals rapidly spread and replaced native populations.

Materials and Methods: We sampled and sequenced whole genomes from 190 individuals across 14 populations in Trinidad and Tobago, including three populations along the Turure River. Samples were sequenced to an average coverage of 15×. Neutral genetic diversity was estimated at four-fold degenerate sites. Genetic load was assessed based on the presence of strongly deleterious loss-of-function (LOF) alleles, milder missense variants and using conservation scores (CS) of derived alleles.

Results: Genetic load was higher in populations from Tobago compared to

Trinidad and increased in upstream sites compared to downstream ones. These patterns were more pronounced for CS-based estimates than for LOF alleles. Consequently, we detected overall significant relationship between neutral genetic diversity and genetic load. However, in the Turure River, we observed contrasting patterns for LOF and CS-based estimates. Strongly deleterious LOF alleles were purged from the Turure population but tended to accumulate along the expansion gradient. In contrast, CS-based load was highest at the introduction site and declined downstream. Furthermore, we detected evidence of increasing admixture along the expansion gradient.

Conclusions: These patterns likely result from an initial population bottleneck and subsequent admixture with native populations. The founder effect led to the loss of deleterious alleles through drift and purging, while relaxed selection allowed mildly deleterious variants to accumulate. However, it appears that as a result of admixture from local populations, LOF alleles are being reintroduced to expanding population while mildly deleterious alleles are being masked. Together, our findings highlight how genetic load in invasive populations is shaped by population reduction followed by admixture, with important implications for understanding biological invasions.



Purging of highly deleterious mutations through an extreme bottleneck

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POSTER SESSION 2 - **P02.078**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background. Transitions to captivity often produce population bottlenecks. On the one hand, bottlenecks increase inbreeding and decrease effective population size, thus increasing extinction risk. On the other hand, elevated homozygosity associated with inbreeding may purge deleterious mutations. Previous empirical studies of purging in captive breeding programs have focused on phenotypic measurements. We test natural selection's ability to purge deleterious mutations following an extreme population bottleneck by analysing patterns of genetic diversity in wild and captive-bred individuals of the Lord Howe Island stick insect, *Dryococelus australis*. *Dryococelus australis* has been bred in captivity for two decades, having passed through an extreme bottleneck—only two mating pairs with few new additions since then. The magnitude of the bottleneck together with high female fecundity but low offspring recruitment set up nearly ideal conditions for the purging of deleterious mutations, but the extremity of the bottleneck also suggests that this effect should be restricted to only the most highly deleterious ones.

Materials and methods. We generated low-coverage whole-genome sequence data of wild and captive *D. australis*, including individuals from a hybrid line which has ancestry from both the original captive breeding program's founders and a wild female collected recently. We estimated individual level heterozygosity and inbreeding as well as group-specific allele frequencies using a genotype-likelihood framework. Using the annotated genome

sequence for *D. australis*, we estimated the fitness effects of segregating mutations by their predicted impact on protein function and compared the frequency of mutations of different classes among wild and captive sample groups. We also sequenced one wild male to high coverage to assess the level of genetic diversity in the wild.

Results. Mean genome-wide diversity in the wild male sequenced to high-coverage was extremely low at $3.6e-4$. The chromosomal landscape of diversity was punctuated by long segments of near-zero heterozygosity indicative of recent inbreeding. Indeed, our estimate of FROH for runs-of-homozygosity > 1 Mb was 0.35. In the low-coverage data, captive-bred individuals had a greater number of long runs of homozygosity compared with wild individuals. Stop-codon mutations were preferentially depleted in captivity compared with other mutations in coding and noncoding regions. The more deleterious a mutation was predicted to be, the more likely it was found outside of runs-of-homozygosity.

Conclusions. Our results strongly support a role for purging of highly deleterious mutations in the landscape of genetic variation in captive *D. australis*, despite the extreme bottleneck and the relaxed selective environment of captivity. This provides a molecular context to the finding that purging may contribute to fitness change in captive bred populations with low N_e and underscores the role of deleterious recessive mutations in fitness variation in small, bottlenecked populations.



Range of trait variation in prey determines evolutionary contributions to predator growth rates

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POSTER SESSION 2 - **P02.061**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Evolutionary and ecological dynamics can occur on similar timescales and thus influence each other. While it has been shown that the relative contribution of ecological and evolutionary change to population dynamics can vary, it still remains unknown what influences these differences. Here, we test whether prey populations with increased variation in their defence and competitiveness traits will have a stronger impact on evolution for predator growth rates. We controlled trait variation by pairing distinct clonal lineages of the green alga *Chlamydomonas reinhardtii* with known traits as prey with the rotifer *Brachionus calyciflorus* as predator and compared those results with a mechanistic model matching the empirical system. We measured the impact of evolution (shift in prey clonal frequency) and ecology (shift in prey population density) for predator growth rate

and its dependency on trait variation using an approach based on a 2-way ANOVA. Our experimental results indicated that higher trait variation, i.e., a greater distance in trait space, increased the relative contribution of prey evolution to predator growth rate over 3–4 predator generations, which was also observed in model simulations spanning longer time periods. In our model, we also observed clone-specific results, where a more competitive undefended prey resulted in a higher evolutionary contribution, independent of the trait distance. Our results suggest that trait combinations and total prey trait variation combine to influence the contribution of evolution to predator population dynamics, and that trait variation can be used to identify and better predict the role of eco-evolutionary dynamics in predator–prey systems.



Reframing the Himalaya: Genomic Insights from 1,217 individuals Into Migration, Isolation, and Malaria Adaptation in a Complex Human Landscape

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POSTER SESSION 2 – P02.066, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The Himalaya, the world's youngest and most dramatic mountain range, is often portrayed as a barrier to migration and a site of extreme ecological adaptation. Yet its broader demographic and adaptive history remains poorly understood. As a biogeographic crossroads and a mosaic of linguistic and cultural diversity, the region offers a rare opportunity to investigate how geography, culture, and deep population history intersect to shape patterns of human genetic variation. Here, we present a comprehensive genome-wide analysis of 1,268 individuals from 60 populations spanning the Himalayan arc—including remote and marginalized groups from Nepal, Bhutan, Tibet, and northeastern India—that expands the focus beyond high-altitude adaptation to encompass the full spectrum of ecological, linguistic, and sociopolitical diversity. By integrating newly generated genotype data with publicly available datasets, we implement local ancestry based tools to reconstruct population structure, admixture history, and patterns of genetic isolation across the Himalayan arc. We identify three distinct sources of East Asian ancestry, including a previously uncharacterized ancient lineage shared by Western Himalayan hunter-gatherers and indigenous Terai groups. South Asian

ancestry entered the Central Himalaya and Terai via at least two pulses—one predating the admixture that formed the ANI-ASI cline, and a second into the Kathmandu Valley. In contrast, Eastern Himalayan groups such as the Kiranti show little South Asian admixture, likely reflecting historical sociopolitical and geographic barriers. In addition, we uncover striking genetic isolation in small, nomadic, or geographically sequestered groups such as the Raute, Ban Rawat, and Toto exhibit extreme homozygosity and minimal gene flow, consistent with long-standing seclusion and endogamy. Beyond demography, we detect strong and replicable signals of local adaptation to malaria in lowland Terai populations, including selection at canonical genes such as AHSP, CR1, AFDN, THSD7B, and ANGPTL1. These findings underscore that infectious diseases—rather than altitude alone—have shaped genetic variation across the Himalayan belt. Taken together, our findings reframe the Himalaya as not just a static barrier and a site of ecological extremity, but as a dynamic frontier of migration, deep ancestral diversity and population continuity that has resulted in cultural complexity, and powerful localized adaptation.



Sexual selection and population spatial structure interact to shape sex-specific evolutionary responses in physiology

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POSTER SESSION 2 - P02.095, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Sexual selection and sexual conflict may lead to sex-specific phenotypic expression of physiological traits because of the different selection pressures acting on females and males. We tested whether population spatial structure modulates the effect of sexual selection on male and female standard metabolic rates and oxidative stress. We used selection lines of the seed beetle *Callosobruchus maculatus* subjected to divergent evolutionary regimes in the intensity of sexual selection (high vs. low, in polygamous vs. enforced monogamous populations, respectively) and the presence of metapopulation structure (absent vs. present), and found that the evolutionary treatments impacted physiological traits in a complex way. In the selection regimes simulating metapopulation structure (i.e.,

divided populations) both sexes had similar metabolic rates; in undivided (unstructured) populations males had lower rates than females. Males from polygamous and undivided populations showed the lowest levels of antioxidant enzymes, resulting in strong sexual dimorphism in antioxidant capacity under this selection regime. The oxidative damage to lipids, instead, was highest for both males and females from monogamous and undivided populations. Our results reveal two key insights. First, physiological traits evolve differently in females and males in response to sexual selection intensity and population spatial structure. Second, such sex-specific physiological responses are linked to selective pressures acting mostly on males.



Speciation from Small Beginnings: Population Size and Environmental Heterogeneity Shape Speciation Dynamics in Individual-Based Simulations

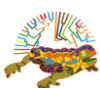
Peacock C¹

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POSTER SESSION 2 - **P02.097**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Understanding the dynamics and drivers surrounding speciation events remains a central goal in evolutionary biology. While classical theory proposes that large, geographically isolated populations are more likely to undergo speciation, which has been documented within real-world investigative studies, recent work which is largely based around simulation models has begun to challenge this view. Elements of alternative mechanisms of speciation, such as sympatry and parapatry, have proven difficult to investigate through conventional field or lab based methods. We utilise an individual-based evolutionary simulation software to investigate how parent species' population size and environmental heterogeneity affect the rates of speciation, community structure, and longevity of daughter species. Using the REvoSim simulation software, we analysed speciation events across multiple runs, with varying levels of environmental heterogeneity and rate of habitat change. Speciation was inferred from phylogenetic trees and speciation logs produced after the completion of each run. We quantified the population size of each parent species at the time of speciation and tracked the longevity

of the resulting daughter lineages, categorising environmental complexity using spatial heterogeneity metrics and rate of environmental change using habitat image refresh rates. Contrary to our expectations, we found that a large proportion of speciation events were skewed towards parent species with relatively small population sizes (less than 5,000 total individuals). These events often initiated rapid diversification clusters - bursts of daughter species emergence within narrow time windows. Further analysis found that the longevity of these clusters correlated positively with environmental heterogeneity, suggesting a stabilising role of more complex habitats. Preliminary spatial analyses suggest that many of these clusters do not align neatly with classical allopatric models, indicating potential parapatric or sympatric dynamics. These findings help to elucidate the role of parent species characteristics and habitat variability on the mode of speciation, providing insight into the mechanisms behind non-allopatric speciation events that are notoriously difficult to study.



Toads in Town: Conservation genetics of increasingly fragmented Western Leopard Toad populations in Urban Cape Town

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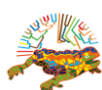
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POSTER SESSION 2 - P02.092, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Understanding the evolutionary and selection dynamics of small, isolated populations in urban areas is crucial for developing effective conservation strategies. This is particularly important in cases where populations still have a viable chance of survival after small-scale conservation interventions. The Western Leopard Toad (*Sclerophrys pantherina*), an endangered species endemic to South Africa's Western Cape, is experiencing significant habitat loss as Cape Town's rapid urban expansion drives increasing human-wildlife conflicts. In this study, we use range-wide genetic data to assess species-wide population structure, connectivity, and gene flow within the species' contemporary landscape. Using 12 microsatellite loci genotyped across 84 individuals, we generated essential biodiversity variables (EBV), constructed spatial genetic networks, and applied estimated effective migration surfaces (EEMS) to investigate gene flow and movement dynamics in the species. Our analyses revealed strong population

structure, identifying three genetic clusters ($K = 3$) within the Western Cape—two of which were located within Cape Town—indicating restricted dispersal over relatively short distances. We identified potential 'genetic reservoir' sites exhibiting strong gene flow within remnant wetlands located at the boundary between urban development and natural habitat in central Cape Town. As well as a population at high risk of extirpation due to office block development. Temporal comparisons of genetic diversity from 2010 to 2021 showed no significant change in genetic diversity, suggesting a potential extinction debt driven by recent, rapid declines in key breeding sites that may not yet be reflected in genetic patterns. These findings highlight the role of human-driven selection in shaping urban species and highlight the importance of integrating genetic data into urban conservation planning to proactively mitigate genetic erosion and prevent vulnerable populations from needlessly reaching critical thresholds.



Topi or not topi: population structure of *Damaliscus lunatus* in Africa and its implication for conservation

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POSTER SESSION 2 - **P02.096**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Africa is currently home to a high number of diverse megafaunas, and topi (*Damaliscus* spp.) is among them. Topi is a member of the Alcelaphinae subfamily of the Bovidae family. The IUCN Red List currently acknowledges topi taxonomy as a conservative arrangement of one species, *Damaliscus lunatus*, which is classified as Least Concern. Currently, topi is divided into six subspecies, *D. l. korrigum*, *D. l. lunatus*, *D. l. superstes*, *D. l. jimela*, *D. l. tiang*, and *D. l. topi* with no clear biogeographical barriers between these populations. Each subspecies is assessed separately by the IUCN with the *D. l. korrigum* and *D. l. tiang* assigned as Endangered, *D. l. jimela* as Vulnerable, and the remaining as Least Concern. There is yet to be genetic support for these assessments, which is substantial for managing taxa with trans-national distribution. We generated 71 whole genome sequencing data of *D. lunatus* populations collected from six countries across eastern Africa, representing populations from the native geographic distribution of *D. l. tiang*, *D. l. jimela*, *D. l. lunatus*, and *D. l. superstes*. Our preliminary results indicate that there were two major clades of *D. lunatus* representing southern topi (Botswana and Zambia) and eastern topi (Tanzania, Kenya,

Ethiopia, Uganda). Admixture analysis revealed a total of nine subpopulations with two coming from the southern area of their distribution. Investigation on their genome-wide variation showed that southern topi has relatively low heterozygosity, especially the ones in Uganda. The effective population size trajectory of the nine subpopulations also suggests that the samples' geographic origins likely represents the subspecies native to that region. Despite the distinct groupings supported by population structure, coalescence-based analysis, and genome-wide variation, gene flow has happened between geographically neighboring populations at a relatively moderate level. To understand whether the nine subpopulations represent distinct evolutionary significant units warrant separate managements, we are assessing their divergence time, genetic load, and conduct selection scans between subpopulations of more than ten individuals to detect local adaptation on separate putative subspecies. This work ultimately aims to elaborate the complexity of designating management units for taxonomically diverse mammals and how different alternatives would impact their long-term survival.



Turnover shapes evolution of birth and death rates

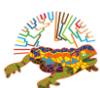
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*POSTER SESSION 2 - **P02.057**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM*

A truly predictive evolutionary theory must be derived self-consistently from the underlying stochastic population dynamics, where the environment and ecology are not treated merely as confounders and mediators of evolutionary dynamics. By explicitly decomposing fitness to its birth and death components as well as accounting for how evolution and ecology respectively might affect them, we show how a fundamental asymmetry between reproduction and survival emerges. First, we derive a stochastic replicator equation from a general birth-death process and demonstrate the importance of the demographic turnover rate (defined as the sum of birth and death rates) on mutant fixation. Then we show how the

turnover rate shapes the distribution of evolutionary trajectories causing a systematic turnover bias in the mutant substitution dynamics in favour of less volatile low-turnover strategies. Finally, we provide theory for predicting how organismal growth strategies evolve in response to different population regulation mechanisms and show how the resulting life-history evolution has a clear direction where the pace of life becomes either slower or faster depending on the ecological context and mutational supply. Overall, our results highlight the importance of demographic turnover in evolution and underline the perils of quantifying fitness with a single parameter.



Understanding the social and genetic intergenerational impact of inbreeding depression in a wild population.

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POSTER SESSION 2 - P02.093, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Understanding the full impact of inbreeding depression is key to informing effective conservation action. However, quantifying it can be complex in wild systems where recording accurate fitness measures and building accurate pedigrees over many generations is extremely difficult. As a result important aspects, such as the intergenerational cost to offspring of having inbred parents, are rarely considered and not fully understood.

Having inbred parents can depress the fitness outcomes of offspring (IID) for various reasons. Inbred social parents may be less able to provide resources and care for offspring, while inbred genetic parents can pass on damaged genetic material. The relative importance of these mechanism is difficult to disentangle. Fortunately, socially monogamous systems where extra pair paternity is frequent can allow us to separate the effect of an inbred genetic father from an inbred social father.

Here, we use exceptionally accurate lifetime fitness data, genomic inbreeding estimates and parentage assignments from >12 generations of a closed wild population of Seychelles warblers, where ca 40% of offspring are extra-pair. In this population IID

has already been identified through preliminary studies, but not well understood. We calculate inbreeding coefficients, we call runs of homozygosity in ~1900 imputed low coverage whole genome sequences. These data allow us to assess the level of IID and disentangle the relative effects of genetic vs social fathers on offspring fitness.

We predict that individuals with higher parental inbreeding coefficients will have shorter lifespans and produce less offspring due to IID. Furthermore, extra pair offspring will have different fitness outcomes depending on the inbreeding coefficients of their social and genetic fathers, thus allowing us to disentangle the relative importance of the mechanisms underpinning IID.

In this presentation I will outline our results and discuss their ramifications. As IID has rarely been examined in wild systems this study will highlight the relevance of finding the full impact of inbreeding. Having a full and nuanced understanding of inbreeding depression is important moving forward as we can only expect it to become more prevalent as populations shrink and fragment under intensifying anthropogenic conditions.



Unraveling the basis of adaptation to divergent habitats in chaffinch populations on the small island of La Palma

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¹National Museum of Natural Sciences (MNCN-CSIC)

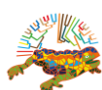
POSTER SESSION 2 - **P02.088**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Studying the evolutionary mechanisms that drive divergence and adaptation in natural populations provides valuable insights into the origin and maintenance of biodiversity and the functioning of biological systems. Oceanic islands, due to their unique characteristics such as limited size and geographical isolation, serve as optimal models for such research field. In this work, we analysed the phenotypic and genomic differentiation among populations of La Palma chaffinches (*Fringilla canariensis palmae*) inhabiting contrasting habitats: humid laurel forest and dry pine forest.

We statistically tested for differentiation in phenotypic traits measured in adult males, including weight, morphometric characteristics and plumage colour. We assessed genetic differentiation between habitats from 250 resequenced whole genomes (20x coverage) using PCA and genome scans of FST. We also conducted Genome–Environment Association (GEA) analyses to detect associations between genetic variation and two ecologically relevant habitat-related environmental variables. Lastly, we performed genome scans to detect signals of selective sweeps on both habitats to identify candidate genes under positive selection.

Our results highlight significant phenotypic differentiation in weight, morphology and plumage colouration across populations and habitat types. We found marked population structure in neutral markers despite the small geographic scale, indicating extremely restricted dispersal within the island. A redundancy analysis revealed clear habitat-related structure, with a separation between cloud and pine forest individuals along the first two RDA axes associated with vegetation greenness and temperature seasonality. Detection of multiple FST peaks of genetic divergence potentially associated to local adaptation and habitat-specific selective sweeps suggests polygenic adaptation. We characterized multiple loci in regions undergoing positive selection, which are involved in tissue development, body size, vision, and melanin pathways, among other functions.

In conclusion, we found evidence for the influence of strong genetic drift, due to small population size and reduced dispersal, a pattern commonly observed in populations colonizing oceanic islands. At the same time, we detected signals of divergence and positive selection associated with the two contrasting habitats, consistent with processes of local adaptation.



A natural experiment in brown trout *Salmo trutta* demonstrates the impact of demography on linked selection

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¹University College Cork, ²Marine Institute, ³Environmental Research Institute

MEETING ROOM 115, August 19, 2025, 2:00 PM - 5:00 PM

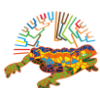
Background. Within and among populations, patterns of genetic diversity are mediated by mutation, migration, drift, selection, and recombination. Recombination interacts with directional selection to reduce genomic diversity by linked selection. Linked selection removes haplotypes that carry deleterious mutations (background selection) or that do not carry beneficial ones (selective sweeps). There are positive correlations in locus-specific nucleotide diversity among closely related species, implying that conserved genomic features (such as conserved recombination rate heterogeneity) shape genetic variation via linked selection at long time scales. The efficacy of this should be modulated by demography, lower N_e populations being subject to greater genetic drift. Here, we test the hypothesis that demography modulates the strength of linked selection by exploiting differences in the degree of connectivity between recently diverged pairs of resident and anadromous populations of brown trout, *Salmo trutta*. Specifically, we hypothesise that (1) nucleotide diversity in 100 kb genomic windows should be positively correlated with local recombination rates in all populations, but (2) the strength of this correlation should be weaker in lower N_e closed resident populations. Additionally, (3) windowed nucleotide diversity should be less similar among pairs of closed resident populations than pairs of open anadromous populations due to the reduction in N_e .

Materials and methods. We generated low-coverage whole-genome sequence data from five resident-anadromous pairs of brown trout populations on the west coast of Ireland. Resident populations living above waterfalls that are impassable to upstream migrants are closed to immigration, whereas

anadromous ones below waterfalls in the same catchment are open. The independent isolation of these resident populations by the formation of waterfalls creates a natural experiment where different local genetic backgrounds are exposed to high (anadromous) and low (resident) N_e . We used a genotype likelihood framework to estimate windowed measures of genetic diversity and recombination rates in each population and compare these estimates across all pairs of populations.

Results. In all populations, there was a significant positive correlation between windowed nucleotide diversity and recombination rates. The slope of this positive relationship was greater for all anadromous populations than any resident population except for the Gweebarra resident population, which we suspect is not fully isolated from its anadromous counterpart. Correlations in windowed nucleotide diversity were greatest for anadromous pairs, weakest for resident pairs, and intermediate for resident-anadromous pairs, even when the resident-anadromous pair occupied the same river.

Conclusions. Overall, our predictions regarding the effects of linked selection were supported, demonstrating that a conserved recombination landscape at the intraspecific level governs the distribution of nucleotide diversity and that this relationship depends on demography. These results have implications for the study of parallel adaptation in divergent populations, as the overlap of responses to a shared selective pressure should depend on the overlap in the amount and kind of standing genetic variation, which in turn is affected by the interaction of recombination rates and demography. Our resident-anadromous population pairs make an excellent model system for these processes.



Adapting to a changing future: Is the 50/500 conservation rule adequate?

Nunney L¹

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MEETING ROOM 115, August 19, 2025, 11:00 AM - 1:00 PM

Background: Global warming is a major threat to biodiversity that goes beyond recent precedent. Historically, many species survived environmental change by shifting their range, but now human-induced habitat loss and fragmentation commonly restricts range shifts, forcing species to adapt in situ or face extinction. While Haldane's "cost of natural selection" estimate, which limited the rate of adaptation to about one genetic substitution per 300 generations, is too conservative, this estimate took no account of any added constraint faced by small populations. These constraints can be examined through simulations that explicitly include environmental change, population dynamics and the frequency of adaptive mutations. Environmental change was assumed to continue for long enough to require novel adaptive mutations.

Materials and methods: Simulations of directional environmental change were performed using a model integrating population dynamics, mutation, the environmental fitness variance of genotypes, and selection. Genetic interactions ranged from additive (one trait) to multiplicative (several traits). The simulations examined the capacity of populations to adapt to an increasing rate of environmental change without going extinct. The model was applied in the conservation context of having either a single panmictic reserve or of a metapopulation of a linked set of smaller reserves. The metapopulation model examined the relationship between adaptation and the level of dispersal between adjacent sites.

Results: Persistence relied crucially on both carrying capacity and the rate of adaptive mutation. Although Haldane's estimate was found to be much too conservative except for the smallest populations, under most conditions a small population of several thousand or less was severely limited in its long-term response to rapid climate change, and vulnerable to extinction. When populations were subdivided, the simulations showed that metapopulations adapt similarly to a panmictic population if gene flow between neighboring reserves exceeds about two effective migrants per generation

Conclusions: Confronted by long-term directional environmental change, a general conclusion is that species unable to shift their range are vulnerable to extinction unless their population size is at least several thousand. However, while big is best, it also needs to be remembered that we cannot generally predict what adaptive challenges a given population may face. Even when a population is smaller than the ideal, any increase in size provides a significant increase in adaptive potential, and this may be enough to make the difference between survival and extinction. In fragmented landscapes, the only option for creating adequate adaptive units is by preserving metapopulations across a network of small reserves connected via habitat linkages. These results argue for significant investment in wildlife corridors, carefully designed to ensure effective function.



Ancient DNA rewrites the evolutionary history of the Western Palearctic robins

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The extinct taxa provide crucial information on biodiversity loss. However, they can also illuminate us about ancient ecological interactions between extinct and extant species; changes in evolutionary history of migration; or how morphological traits can rapidly evolve. With the present study, we revealed several new extinct taxa found in the Atlantic oceanic archipelago of Azores. The cranial morphology of some of them was so different to any other species known that it deserved the common name of “Enigma”. We retrieved ancient DNA from some Enigma remains found in the island of Graciosa (Azores); and phylogenetic inferences unambiguously determine their phylogenetic positions. Both extinct taxa were grouped within the Muscicapidae family. One individual bone was strikingly grouped within the extant Canarian robin clade composed by *Erithacus superbis* and *E. marionae*. The

remaining bones sequenced were grouped in a new clade of its own. This new clade was sister to all Eurasian robins (*Erithacus* spp). The Enigma and the genus *Erithacus* shared a common ancestor 2.5-3.5 millions of years ago, and such age should be considered as the time of colonisation in Azores. Overall, our results show: 1) the arrival of robins and allied species in Azores, and Macaronesia, was the result of, at least, three independent bouts of colonisation in a relatively recent age (< 4 my); 2) in Azores co-occurred in sympatry two contemporary robin species plus a new morphologically distinct taxon named “Enigma”, however, only the extant European robin survived to human arrival; and 3) the unique morphology displayed by “Enigma” is likely a consequence of a rapid morphological change based on the age of divergence between the mitogenomes of the new taxon and modern robins.



Coping with insularity: genome-wide effects of isolation and genetic drift in highly diverse primates.

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How do small, more isolated, populations of wide-spread genetically diverse species buffer against genetic diversity loss and genomic load? Insular populations persist at low population sizes, with lower levels of genetic diversity, and higher inbreeding than their mainland counterparts. Despite the potential for harmful genetic diversity loss, many populations remain stable and the genetic mechanisms that support this remain unclear. The spot-nosed monkey (*Cercopithecus petaurista*), the Campbell's monkey (*Cercopithecus campbelli*) and the green monkey (*Chlorocebus sabaeus*) are widespread and genetically diverse West African primates. Insular populations can be found at the Bijagós Archipelago, Guinea-Bissau. Here, we assessed the genetic diversity, inbreeding, genetic load and adaptive variation using whole genome sequencing data from insular and mainland

populations. In the three species, insular populations showed ~15 – 50% reduction in genome-wide diversity and ~70 – 90% higher proportion of the genome in ROH than mainland populations. Genetic drift has likely promoted the conversion of masked genetic load into realised load. However, we found no evidence for elevated deleterious variation in the insular populations nor increased rates purging. Species-specific traits, including high extant genetic diversity and ecological flexibility, likely support this phenomena. Despite their current stability, novel anthropogenic factors are increasingly threatening these insular populations and may interact negatively with the observed increase in realised load. Conservation management is advised to safeguard their remaining genetic diversity.



Developmental temperature, not inbreeding, shape life history and locomotor behaviors in juvenile guppies (*Poecilia reticulata*)

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Environmental and genetic factors play a critical role in shaping life-history traits and behavior, yet their combined effects remain underexplored. Small, isolated populations are particularly susceptible to inbreeding, which can alter developmental trajectories and fitness-related traits. We examined how developmental temperature and inbreeding influence key life-history traits (early growth rate, size and age at maturity, survival, and sex ratio) and juvenile locomotor behaviors (boldness and habituation) in guppies (*Poecilia reticulata*). Guppies are live-bearing fish that typically inhabit shallow streams, which frequently fragment into isolated pools during the dry season, leading to inbreeding in natural populations. This makes them an ideal system to examine how environmental and genetic factors interact to shape life history and behavior traits in small population. Using a controlled breeding design, we generated inbred and outbred individuals that were reared at either a control temperature (26°C) or an elevated temperature (30°C) from birth until maturity. The developmental temperature strongly affected several life-history traits. Both sexes

matured earlier at 30°C, but only males exhibited slower early growth and reduced size at maturity, while female growth and size at maturity remained unaffected. Similarly, higher developmental temperature reduced the boldness of juveniles in both sexes; however, only females took longer to habituate to a novel environment. In contrast, inbreeding had no significant effects on any of the measured traits, nor did inbreeding interact with temperature. Our results indicate that, under our experimental conditions, developmental temperature was a driver of phenotypic plasticity in guppies, generating distinct sex-specific trade-offs in both life history and behavior. While inbreeding is expected to reduce fitness in small, isolated populations, our results suggest that its effects may not be universal and could vary depending on environmental conditions and the specific traits being measured. Such findings underscore the pivotal role of thermal and genetic environments in shaping developmental trajectories—an important factor to consider as the climate continues to change.



Dispersal evolution as a driver of island biodiversity

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The origin of island biodiversity is thought to lie in the isolation that results from this peculiar geography. Several theories have been proposed to explain the underlying processes responsible for speciation, including dispersal theory. However, the role of dispersal in shaping patterns of biodiversity seems paradoxical: it induces speciation by providing individuals with new opportunities but inhibits speciation by allowing gene flow. We investigate whether this paradox can be resolved by considering dispersal as an evolving trait, given that dispersal is known to be under selective pressure. In addition, given a stable species richness in a system: is this the result of a low speciation rate or high speciation and

extinction rates balancing each other out? We test this using an individual-based model that mimics an archipelago system and compare this to a neutral landscape. We find that certain dispersal patterns are linked to specific island characteristics such as size and isolation, and prominent dispersal evolution is observed in approximately 10% of speciation events. However, dispersal evolution is not the sole driver of speciation: multiple speciation processes co-occur in the same landscape. Different mechanisms underlie the stable species richness for the different scenarios: speciation and extinction are rare in a heterogeneous landscape, whereas they cancel each other out in a neutral landscape.



Extreme climatic event causes genetic bottlenecks across a wild butterfly metapopulation: genomic consequences for population adaptative potential

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Background: Climate change can exacerbate already poor habitat quality of small populations residing in fragmented landscapes. As many such populations will need to genetically adapt to persist, it is crucial to assess the drivers and constraints underlying their adaptive potential. We used temporal genomics to investigate the interplay between habitat fragmentation and climate change in a Finnish metapopulation of the Glanville fritillary butterfly (*Melitaea cinxia*).

Materials and methods: Utilizing a unique long-term sample collection from the last 15 years, we assessed how population fluctuations between years – resulting from the system’s inherent local extinctions and recolonizations dynamics – impact the effectiveness of selection in driving genetic change over time. To do so, we generated a high-resolution temporal genomics dataset, whole-genome sequencing 506 individuals from six replicate networks across 8 different timepoints, and used time-series and genotype-by-environment analysis to find adaptive genomic changes over time.

Results: Our time-series analysis showed that an extreme summer drought in 2018 not only resulted in a 10-fold decline in population sizes at the demographic level, but that this extreme climatic event also had severe genomic consequences in the form of genetic bottlenecks across the replicate sites. Effective population sizes across the networks were small (36-63 individuals) with strong drift resulting in genome-wide allele frequency changes over time. By combining genomic diversity and inbreeding measures with genotype-by-environment analysis, simulations, and a candidate gene approach, we discuss evidence for signatures of selection despite this strong drift.

Conclusions: Our genomic characterization of metapopulation eco-evolutionary dynamics highlights how an extreme climatic event can impact population adaptive potential, providing important insights into what determines the rate of genetic adaptation in small populations.



Fighting Through the Heat: How Sexual Selection Shapes Small Population Demography Under Recurrent Heatwaves

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Sexual selection is a potent evolutionary force with complex effects. Strong sexual selection can enhance adaptation and reduce mutational load, while simultaneously reducing survival, or causing sexual conflict that reduces fitness for one or both sexes. Many populations today face not only gradual environmental changes but also extreme, short-term stress events like droughts or heatwaves. The combined effects of sexual and environmental selection on population demography during and after such events remain poorly understood, even though such combined effects could be crucial for the persistence of small, endangered populations under climate change. In this study, we investigated how sexual selection affects survival during environmental stress by manipulating the expression of an aggressive fighter morph in small populations of the male-dimorphic soil mite *Sancassania berlesei*, exposing some of these populations to recurrent periods of extreme heat and monitoring survival over eight generations. We found that heat exposure reduced survival, more severely in females

than in males, and survival was lower in populations with higher fighter prevalence, but there was no interaction between temperature and fighter morph prevalence. Furthermore, survival declined across generations, and the decline was steeper in populations with lower prevalence of fighters, leading to the loss of their initial survival advantage by the last generation. Three populations exposed to heat became extinct during the experiment, all from the reduced fighter expression treatment. Our findings imply that despite its cost to individual survival, sexual selection does not modulate population sensitivity to heatwaves over several generations. Furthermore, we demonstrate that these mortality costs of sexual selection are gradually compensated over successive generations, which could be a result of a more effective purging of inbreeding depression. Thus, while the additive effect of sexual selection and heatwaves on survival may increase demographic risks for small populations in the short term, sexual selection may increase resilience of populations to prolonged bottlenecks.



Genetic and dental variation: The effects of small population size in the endangered Saimaa ringed seal

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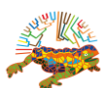
Background: Genetic diversity is considered the raw material for natural selection and consequently for adaptation. Because genetic diversity is often higher in larger populations, it is widely assumed that larger populations also harbor more phenotypic variation in ecologically relevant traits and can better adapt to changes in their environment. Meanwhile, smaller populations might suffer from increased frequencies of maladaptive phenotypes. However, this hypothesis has limited support in wild populations. Here, we address the connection between population size, genetic diversity and variation in an ecologically important trait – tooth shape – using genomic and dental variation data from four ringed seal populations (Arctic Ocean, Baltic Sea, Lake Ladoga, Lake Saimaa) with greatly differing population sizes. The smallest of these populations is the endangered, land-locked and bottlenecked Saimaa ringed seal, with a census population size of less than 500 individuals, and the largest is the Arctic ringed seal population, with a vast geographic range and millions of individuals.

Materials and methods: We calculated nucleotide diversity (π) and derived allele frequencies using whole-genome sequencing (WGS) data from 86 ringed seal samples. We then measured dental diversity and the occurrence of tooth anomalies in the same populations. Specifically, we calculated the number of dental cusps for postcanines 2-5 in 150 seals per population and defined dental anomalies as the presence of enlarged or extra teeth. For the latter, we carried out genome-wide association (GWA) mapping using WGS data from 93 Saimaa ringed seals phenotyped for the anomaly.

Results: As hypothesized, our analyses showed both a higher average nucleotide diversity and a higher overall number of genetic variants, especially rare ones, in larger populations

compared to small ones. The average full-genome nucleotide diversity was 0.0031 for the Arctic population, 0.0029 for Baltic, 0.0027 for Ladoga and 0.0015 for Saimaa. Thus, nucleotide diversity varied by a factor of two between Lake Saimaa and the Arctic population. Allele frequency comparisons showed similar trends, with the number of singletons in the Arctic population (5822120) being an order of magnitude higher than in the Saimaa population (479051). Overall, the number of segregating biallelic SNPs in the Arctic population was three times that of the Saimaa population. Consistent with the genomic data, the phenotypic data support higher dental diversity in larger populations. The morphological disparity was 8.2 for the Arctic population, 7.4 for Baltic, 6.6 for Ladoga and 5.4 for Saimaa. Lack of rare morphotypes in the Saimaa population may be driven by a lower number or rare genetic variants or allele combinations, although adaptive changes due to a long isolation history may also be a factor. In contrast to the non-anomalous variation, we observed a higher frequency of anomalous dental variation in smaller populations compared to large ones (13.5% in Saimaa, 1.8% in the Arctic). Phenotype-genotype mapping revealed a ~140 kb wide candidate region associated with the phenotype. The region is located close to a gene essential for tooth development, *Bmpr1a*, and explains 4.2% of the phenotypic variation.

Conclusions: Our results support the theory of population genetics stating that larger, more genetically diverse populations also harbor more phenotypic variation in ecologically relevant traits, potentially indicating greater adaptive potential. However, anomalous dental variation with a suggested genetic component was more frequent in small populations, possibly due to the accumulation of these variants through reduced selection efficacy and increased genetic drift.



Investigating mutation load associated with adaptation in threespine stickleback

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Positive selection causes beneficial alleles to rapidly rise to high frequency in a population. This can cause linked genetic variation to “hitchhike”, and thereby also rise in frequency. This linked variation may include deleterious recessive alleles, previously neutrally harbored in heterozygous genotypes at low frequency. Modeling studies have shown that local effective population size and reduced recombination rate should contribute to the probability of deleterious mutations being swept to high frequency by being linked to beneficial alleles in selective sweeps or inversions. Sticklebacks provide an excellent system for understanding recurrent evolution. Marine sticklebacks have repeatedly adapted to thousands of freshwater habitats that became available after the last ice age, resulting in the formation of distinct marine and freshwater ecotypes. Upon colonization of new freshwater habitats, selection acts on ancient standing genetic variation present in

marine populations, causing freshwater-adaptive alleles to increase rapidly over tens of generations. These genomic regions play a key role in the repeated freshwater adaptation of morphological, physiological, and behavioral traits, and evolve under strong selection. We find that Norwegian lake populations with small effective population sizes share the core EDA haplotype containing freshwater-adaptive alleles. However, the flanking regions of this haplotype are highly differentiated between different lake populations, suggesting that different haplotypes have hitchhiked with the core EDA haplotype in each lake. We estimate the mutation load in regions of low recombination, including inversions, and the EDA haplotype. We find evidence for variation in hitchhiking mutation load among genomic regions and populations, suggesting a role for both local demographic history and genomic architecture. We highlight how this process could be more widespread in nature.



Phenotypic and genomic impacts of using inbred versus outbred rescuers in the experimental genetic rescue of small, inbred populations

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Anthropogenic change is causing many natural populations to become small and isolated, often leading to inbreeding depression. Genetic rescue aims to alleviate this problem by reintroducing genetic variation. However, the best source of such rescuers, and how that may impact the phenotypic and genomic consequences of genetic rescue, is not fully understood. For example, the traditional view recommends using individuals from a closely related, outbred population to rescue an inbred population. However, such outbred populations will likely harbour many deleterious alleles (masked genetic load), which will be introduced into the inbred populations. Such alleles may then become expressed in the recipient population in later generations if that population remains small. Therefore, it has been argued that using rescuers from closely related but different inbred populations, which should have purged more of their genetic load, may be better. We used *Tribolium castaneum* to experimentally test how productivity and

genomic characteristics differed in inbred populations rescued with individuals from different backgrounds. Our results show that outbred rescuers improved productivity the most, but rescuers from inbred populations still led to some degree of genetic rescue. In terms of the recipient population's genomic characteristics, outbred rescuers reduced inbreeding/RoH in the recipient population but inbred rescuers did not. Outbred rescuers did introduce more deleterious mutations, however, a greater proportion of deleterious alleles were in the heterozygous state in those population (i.e. the populations had lower expressed genetic load) than the populations rescued by inbred individuals. We also present results related to the use of rescuers from populations previously evolved under sexual selection versus monogamous regimes. Finally, we will discuss the importance of monitoring the downstream evolution of such rescued populations over many generations to fully understand the impact of the genetic rescue of small populations



Rapid vs. Slow Habitat Loss: Species Traits Shape Genetic Diversity Patterns During Contraction

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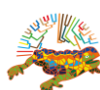
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Species ranges are dynamic, experiencing expansions, contractions, or shifts in response to habitat changes driven by climate change and human activities. Many species have recently experienced population decline, which, under a simplified theoretical model, is expected to reduce their genetic diversity over time. While some empirical studies support this prediction, others report that genetic diversity can be maintained or even increased despite a drastic decline. Here, we investigated how the dynamics of genetic diversity vary among species with different traits (such as generation length, population density, and dispersal) when subjected to a similar scenario of habitat expansion and contraction over millennia. Using both spatial simulations and a simpler panmictic population model, we investigated the temporal genetic diversity in refugium populations undergoing habitat expansion, followed by stationary and contraction periods. By varying habitat contraction speed

and species traits, we identified three temporal dynamics of genetic diversity during habitat loss: (i) a continuous decrease, (ii) a transient increase followed by a decrease, and (iii) a continuous increase. These different genetic diversity trajectories during habitat contraction help explain why rapid contractions did not consistently preserve more diversity than slower ones. More generally, we found varied effects of contraction speed on genetic diversity levels at the end of contraction. Specifically, we revealed a non-linear relationship: intermediate contraction speeds preserve greater genetic diversity than fast or slow contractions. This challenges the belief that high genetic diversity in a refugium population is due to a recent and rapid habitat loss. Overall, we demonstrate that genetic diversity patterns during habitat contraction depend on the interaction between species-specific traits and the dynamics of habitat change.



Recombination rate and efficiency of linked selection in small and large stickleback populations

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Population genetic theory predicts that natural selection will be more efficient in large than small populations because genetic drift reduces efficiency of selection in small populations. Small populations adapting to new environments can also be expected to evolve higher recombination rates to facilitate adaptation as well as to dissociate and purge harmful mutations. We tested these hypotheses (1) by investigating differences in the strength of association between nucleotide diversity (π) and recombination rate across the genomes of nine-spined sticklebacks (*Pungitius pungitius*) from four small pond and four large marine populations, as well as (2) by comparing recombination rates between

small and large populations using population specific linkage maps. We found the predicted positive correlation of π with recombination rate from all but the smallest pond populations suggesting prevalent purifying selection even after accounting for variation in GC/CpG content, gene density, and mutation rate. Mean recombination rates were significantly higher in the pond than in the marine populations. These results suggest an important role for linked selection in reducing π in low recombination regions of both small and large populations. Moreover, as predicted by theory, pond populations appeared to have also evolved higher recombination rates than their marine ancestors.



Sky-Islands under pressure: how geography, climate, and genomic shape the history of an endemic Pyrenean species

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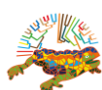
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Understanding how small and spatially isolated populations respond to environmental change is a key challenge in evolutionary biology, with important implications for biodiversity conservation. High-mountain ecosystems, often fragmented into sky-islands, provide natural laboratories to explore these dynamics. Here, we focus on *Xatartia scabra*, a narrow endemic plant restricted to the eastern Pyrenees and the sole representative of a monotypic genus, to investigate how geography, demography, and environment have shaped its genetic structure.

Using a population genomics approach based on RAD-sequencing, we characterized genome-wide SNP variation across eleven populations sampled throughout the species' known range. Given the large genome size of *X. scabra*, RAD-seq provided an efficient and cost-effective alternative to whole-genome sequencing, enabling high-resolution insights into population structure and history. We combined these genomic data with species distribution modeling to reconstruct its geodemographic history and assess the role of past climate fluctuations in shaping current patterns of diversity and connectivity.

We found that genetic structure closely mirrors mountain summit distribution, supporting the hypothesis of persistent isolation among sky-island populations,

likely driven by topographic and ecological constraints. Despite this, traces of admixture between geographically neighboring populations suggest intermittent connectivity, possibly during past climatic optimum. These findings point to a complex demographic history involving both prolonged isolation and episodic gene flow. By integrating climatic variables, we further explore the potential for environment-driven genomic divergence. The association of some populations with temperature regimes and others with precipitation patterns suggests that environmental heterogeneity among isolated summits may contribute to genomic divergence through isolation-by-environment and possible local adaptation. Together, our results illustrate how long-term geographic isolation, demographic dynamics, and environmental heterogeneity jointly shaped the genomic landscape of this endemic species. By integrating population genomics with ecological modeling, we offer a comprehensive perspective on the geodemographic and genetic history of small, sky-island populations. It also raises important questions about the resilience of such taxa under future climate scenarios, emphasizing the need for conservation strategies that consider both patterns of genetic connectivity and environmental specificity.



Small populations and disease: Genomics of a severely bottlenecked species, the Critically Endangered Bellinger River Turtle (*Myuchelys georgesi*)

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Small, isolated populations are highly vulnerable to disease outbreaks due to genetic diversity loss. The Critically Endangered Bellinger River Turtle (*Myuchelys georgesi*) is a long-lived species endemic to a single river in eastern Australia, which suffered a severe population decline in 2015 following a novel disease outbreak. We conducted genome-wide and functional gene analyses to examine how small population size, genetic drift, and the 2015 population crash have influenced the contemporary genetic diversity of the species. To do this we generated a chromosome-level reference genome for *M. georgesi* and analysed 31 re-sequenced genomes to assess genome-wide and immunogenetic diversity within the major histocompatibility complex (MHC) from before ($N = 19$) and after ($N = 12$) the disease outbreak. We also undertook demographic history analyses to investigate temporal changes in effective population size. Genome-wide diversity in *M. georgesi* was critically low, with 90% of the genome within either a long or short run of homozygosity (ROH). However, the number of SNP variants within the functionally important MHC region

and across chromosome 10, exceeded that of all other macrochromosomes ($N = 9$). Additionally, we found the structure of the MHC core region to be highly conserved, a feature that has not been previously characterised in testudines. There were no significant shifts in genomic or MHC diversity from before and after the population crash, potentially due to genetic signatures of selection requiring multiple generations to show in this long-lived species. PSMC analyses revealed prolonged declines in effective population size, likely the result of historical bottlenecks associated with the species' isolated demography, which are reflected in the high prevalence of ROH. These genomic insights reveal how demographic events such as historical isolation and bottlenecks shape contemporary diversity patterns and may influence the adaptability of populations to anthropogenic threats, such as novel disease outbreaks. Additionally, our work highlights the growing importance of understanding genetics to support a multidisciplinary approach to conservation management and long-term viability of small, single-species populations.



Using proxies of genetic load to guide conservation management: do they deliver what they promise?

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A central question in conservation is how to manage threatened populations to improve their chances of long-term persistence. Small, genetically isolated populations risk extinction from inbreeding depression, loss of genetic variation and increased genetic load. The classic approach of minimizing inbreeding and increasing genetic diversity—by augmenting gene flow into genetically eroded populations—is effective at alleviating inbreeding depression and improving population fitness and growth. Recently, however, concerns arose about overlooking the effects of harmful genetic variation on fitness (i.e. genetic load). Because of the difficulty of measuring fitness in wild populations, methods that quantify genetic load focus on identifying putative deleterious variation, which is summarized into individual- and population-level measures. These proxies of genetic load are then used to infer the fitness of an individual (or population), and whether a population has undergone purging. One such method is GERP, which quantifies mutations' presumed harmfulness by identifying the level of evolutionary conservation of the loci in which they occurred. Despite the growing use of genetic load proxies to guide genetic management, the fundamental assumption that managing genetic load will lead to better conservation outcomes—because genetic load proxies are superior fitness predictors—has yet to be thoroughly tested. Here, we combined individual fitness and genomic data of the yellow-tufted honeyeater (*Lichenostomus melanops*) to test the key

assumption of genetic load studies of wild populations: that genetic load proxies predict individual fitness better than inbreeding metrics do. We reproduced 25 GERP-based individual genetic load proxies, and explored their association with lifetime reproductive success (LRS) of 43 wild birds. We also calculated inbreeding coefficients more typically used in genetic management: whole-genome homozygosity (FHOM), and Runs of Homozygosity (FROH). We found that although proxies of realised load predicted individual LRS to some degree, they were not the best predictors: the best inbreeding coefficient predicted LRS 21.1% better than the best genetic load proxy did. Additionally, total load proxies gave conflicting signals of whether *L. m. cassidix* experienced purging: four proxies indicated purging, three suggested accumulation of deleterious alleles, and five showed no differences. Our results suggest that caution is warranted when drawing conclusions about purging, until genetic load tools are validated and best practices are established. As more genomic data become available, more studies from wild populations with lifetime fitness data are needed to validate and benchmark different genetic load proxies. So far, there is no evidence to support the use of genetic load proxies instead of inbreeding coefficients to guide conservation programs. Wildlife management where load has been assumed to be the best predictor of fitness, or purging has been inferred, should be revisited in light of this growing evidence.



Variation among neighbouring wild populations of *Nicrophorus vespilloides* in mutation load and dependence on parental care

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MEETING ROOM 115, August 19, 2025, 2:00 PM - 5:00 PM

Background: Although a substantial body of work has investigated the adaptive value of social behaviour, the contribution of social behaviour to local evolutionary processes remains underexplored. Yet this is key to understanding how quickly populations can adapt in a changing and fragmenting world. Previous research on lab populations of burying beetles (*Nicrophorus vespilloides*) revealed that post-hatching parental care can promote greater standing genetic variation and buffer against inbreeding depression. This means that, in principle, parental care can become evolutionarily self-reinforcing because it compensates for the negative effect of the increased mutation load it allows to build up. We investigated whether there is evidence to support this hypothesis using wild populations of *N. vespilloides*. We investigated whether six neighbouring wild populations vary in their resilience to inbreeding and in the extent to which larvae are dependent on parental care to survive.

Materials and Methods: Burying beetles provide elaborate biparental care for their larvae, which involves converting small vertebrate carrion into an edible nest for larvae, defending the nest and brood from attack and provisioning larvae with fluids through oral trophallaxis. Larvae are facultatively dependent on post-hatching care: they can survive without care in the lab though subsequently mature into lower quality adults. We investigated whether there is variation among six neighbouring wild populations, from ancient woodlands on the Cambridgeshire / Bedfordshire border, in the extent of larval dependence on parental care, and whether this is associated with mutation load (assessed by measuring inbreeding depression). Historical ecological analyses indicate that these distinct ancient woodlands are probably fragments of

the primeval Wild Wood that once covered most of England.

Each wild-caught population was bred in the lab in a common garden environment for at least three generations to eliminate any residual maternal and environmental effects. Each population was then split into four treatment groups: (1) Outbred with full care (control), (2) Outbred with no care, (3) Inbred with full care, and (4) Inbred with no care. To inbreed, we paired siblings. In the no care treatments, the parents were removed after preparation of the nest was complete but before any eggs hatched. Removing care in the inbred treatment allowed us to indirectly measure mutation load by observing inbreeding depression.

Results: Preliminary results indicate that these neighbouring wild populations vary in the degree of inbreeding depression, as assessed by comparing treatment (4) across populations. Furthermore, comparisons of treatments (1) and (2) across populations indicate that populations vary in the extent to which larvae are dependent on parental care to survive. Finally, comparisons of treatments (3) and (4) show that parental care buffers against inbreeding depression in all wild populations.

Conclusions: Even though these neighbouring wild populations of *N. vespilloides* are only between 1 and 10 km apart, they have apparently diverged in their dependence on post-hatching parental care and in their resilience to inbreeding. The challenge for future work is to test whether these factors are causally related, and to determine whether other factors might also affect mutation load - such as population size and heterospecific competition. This work will shed light on the ways social behaviour can influence the rate and direction of local evolution in a rapidly changing and fragmenting landscape.

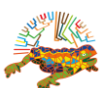




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EVOLUTIONARY BIOLOGY

**S17 - Evolution of behavioural diversity:
from ecology to genes and neural
systems**



Adaptive plasticity in escape behaviour: Crickets don't run as fast as they can when facing predator threat

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POSTER SESSION 3 - **P03.034**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Predator-prey dynamics impose strong selective pressures on the evolution of anti-predator strategies, which constitute a fundamental adaptive response in wild animals. However, these strategies incur energetic costs, requiring trade-offs with other fitness-related traits. While predator-prey interactions have been extensively studied, empirical evidence for behavioural plasticity in responses across predation risk gradients remains limited.

We conducted field experiments on wild cricket (*Gryllus campestris*) nymphs in northern Spain, to investigate (1) how the intensity of predation cues influences escape speed adjustments and (2) whether anti-predator strategies vary by sex, developmental stage and according to

thermal conditions. We simulated two levels of vibrational cues: low-intensity and high-intensity. Contrary to our threat-sensitivity predictions, nymphs exhibited slower escape responses to high-intensity cues. This pattern may reflect ecological relevance: Low-intensity cues indicate immediate threats from avian or reptilian predators whereas high-intensity stimuli might resemble non-lethal disturbances from larger mammals or human activity.

Consistent with our previous findings in adults, escape speed in nymphs increased with body temperature. However, sex-dependent variation was observed only in adults, not in nymphs, suggesting developmental differences in anti-predator strategies.



Alpha and Beta Diversity of Cultural Traits in Global Jukebox Cantometrics Data

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¹Charles University, ²Center for Theoretical Study

*POSTER SESSION 3 - **P03.038**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM*

This study applies the ecological concepts of alpha and beta diversity to cultural traits. The goal is to explore how cultural traits vary within and between neighbouring groups of communities, and to evaluate the usefulness of these frameworks in studying cultural diversity. Alpha diversity metrics measures within-group variation, while beta diversity assesses differences between groups. As an example we use cultural data from the Global Jukebox dataset, a collection of traditional music along with cantometric song characteristics. Geographic and cultural patterns of diversity are investigated to reveal trends. The application of alpha and

beta diversity metrics demonstrates their potential as effective tools for analyzing cultural traits. These frameworks provide a structured way to quantify within-group variation and between-group differences, revealing patterns in the Global Jukebox dataset that align with expectations of cultural diversity studies. This study highlights the potential of applying ecological diversity concepts to cultural research offering a quantitative, scalable, and comparative approach to cultural variation and contributing to wider discussions on cultural evolution and diversity.



Arms-race dynamics drive rapid behavioural coevolution in a parasitoid fly and its host in Hawaii.

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POSTER SESSION 3 - P03.032, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Host-parasite interactions are predicted to drive cycles of adaptation and counter-adaptation, but little is known about the genomics of behavioural co-evolution in animal systems. I will present work that identifies the genomic signature of behavioural coevolution during rapid arms-race dynamics in the wild in Hawaiian insects: an eavesdropping parasitoid fly (*Ormia ochracea*) attacks a field cricket (*Teleogryllus oceanicus*) that recently and adaptively lost its acoustic sexual signal. Across its native North American range, the fly uses a novel hearing organ to detect the sexual signals of crickets and katydids, but over the past 20 years, multiple male-silencing and song-reduction phenotypes have spread in Hawaiian *T. oceanicus*—convergent adaptive responses to escape parasitism. We identified genomic

signatures of counter-selection on flies imposed by rapid host defence evolution using ~350 fly whole-genome sequences and replicate field experiments. Using gene-environment association tests in which the fly's environment consisted of host adaptations present in each of 11 sampled Hawaiian focal populations, we found that strong selective sweeps in *O. ochracea* underlie rapid counter-adaptation to parasitise silent *T. oceanicus*. This occurred despite a strong population bottleneck following the fly's introduction into Hawai'i only ~50 years ago. Thus, sweeps are linked to local selection on the fly imposed by host adaptation to the fly itself. Our study reveals how genomic arms-race dynamics can drive rapid behavioural coevolution in natural host-parasite systems with prominent founder effects.



Comparing the sex-specific demographic history of primates with contrasting social systems

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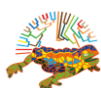
¹Department of Medicine and Life Sciences, Institute of Evolutionary Biology (UPF-CSIC), Universitat Pompeu Fabra, ²Centro Nacional de Análisis Genómico (CNAG), ³Institut Català de Paleontologia Miquel Crusafont, Universitat Autònoma de Barcelona, ⁴Institució Catalana de Recerca i Estudis Avançats (ICREA), ⁵Baylor College of Medicine, ⁶Illumina Inc.

POSTER SESSION 3 - P03.051, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Non-human primates exhibit a wide variety of social systems, reflected in group composition, mating systems and dispersal behaviour. Depending on the social system, groups may exhibit unbalanced sex ratios or sex-biased reproductive skew. These social characteristics are expected to leave detectable genetic signatures, particularly on uniparentally inherited markers such as the Y chromosome and mitochondrial DNA (mtDNA), as well as on the X chromosome. For example, male-biased dispersal may increase Y-chromosome diversity within groups, while polygynous systems, where a limited number of males monopolize access to multiple females, are expected to reduce Y-chromosome genetic diversity relative to mtDNA genetic diversity. Although these genetic signatures have been highlighted in several species, the respective roles of group organisation, mating system and dispersal system in shaping uniparental and X-chromosome genetic diversity remain to be explored. Additionally, changes in social systems over time could also be traced through their genetic footprints. In humans, shifts in kinship systems towards patrilineal organisations following the Neolithic transition could have resulted in the observed reduction of Y chromosome diversity and male effective population size around 5000-3000 years ago.

In this study, we use a dataset of high-quality, whole-genome sequences from 19 primate species to investigate patterns of genetic diversity in uniparental markers, X chromosomes, and autosomes. We aim to assess whether these patterns correlate with known social systems, and whether shifts in these social systems can be identified through Bayesian inference of sex-specific demographic histories. One major challenge is the absence of assembled Y chromosomes in many primate reference genomes. To address this, we use a method based on differential coverage between females and males to identify and extract sequences from the Y chromosome, as well as from mtDNA, X chromosomes, and autosomes. This approach allows us to reliably genotype sex-specific markers even in the absence of fully resolved sex chromosomes, providing a robust dataset for demographic inference.

By comparing sex-specific demographic histories across species, we aim to increase our knowledge of the links between genetic patterns and group organisation, mating systems or dispersal strategies. Furthermore, our approach allows us to explore within-species variation and flexibility in social systems, and to detect potential historical transitions in these social systems.



Convergent patterns of visual adaptation in a community of mimetic butterflies

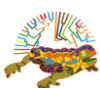
Wainwright B¹

¹University Of St Andrews

*POSTER SESSION 3 - **P03.043**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM*

Within complex tropical rainforests, niche partitioning can expose species to distinct sensory realms. For many animals, visual systems are the primary sensory conduit for interacting with the external world, but little is known of the role light variation plays in strengthening adaptive species assemblages and driving predictable patterns of visual system evolution within rainforests. By studying a community of 54 mimetic butterfly species from a diverse

Neotropical radiation, we take a comparative approach to demonstrate that rainforest architecture can create light microhabitats which drive sensory convergence towards distinct adaptive optima, at the perceptual and processing level. Our study offers a mechanistic insight into the evolvability of visual systems when confronted with similar ecological challenges and the wide-reaching indirect consequences of mutualistic mimicry on trait evolution.



Dissecting the genomics of migration in the Painted Lady butterfly

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POSTER SESSION 3 - P03.046, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Insect migration is a multifaceted trait encompassing morphological, behavioural and physiological adaptations to environmental seasonality. While such complex traits are often underpinned by highly polygenic architectures with numerous variants distributed across the genome, some are shaped by discrete genomic regions with large effects. Insects exhibit a remarkable diversity in their movement types—ranging from full migration to sedentary lifestyles—which shapes connectivity and, ultimately, genetic diversity within species. This variation, apparent even within species, provides a valuable framework to investigate the genomic basis of migration. The migratory Painted Lady butterfly (*Vanessa cardui*) exemplifies this intraspecific diversity, exhibiting differences in migratory timing, orientation, and, in some populations, potential migratory loss.

In this study, we examine how migratory dynamics shape genetic variation across the nearly global distribution of *V. cardui*. By analysing 358 genomes, we test whether populations from two tropical island

archipelagos—Indonesia and Hawaii—have independently adapted to a sedentary lifestyle, a phenomenon known as "migratory drop-off." In contrast, we also investigate whether populations in the Northern and Southern Hemispheres have evolved distinct migratory strategies in the light of opposing seasonal regimes, a mechanism we term "hemisphericity."

Our results reveal widespread genomic signatures associated with migration loss in island populations, consistent with polygenic selection acting on numerous small-effect variants. Conversely, we uncover a 9 Mb chromosomal inversion differentiating mainland Northern and Southern hemisphere populations, pointing to a modular genomic architecture underpinning alternative migratory strategies at each hemisphere. Finally, we characterize genes and regulatory pathways linked to migration, including loci related to neuronal and hormonal regulation. Together, our findings illuminate the diversity of genetic architectures of insect migration and offer insight into the evolution of complex adaptive traits in natural populations.



Does it pay to be picky? How local adaptation in nesting behavior influences offspring phenotypes in a reptile with environmental sex determination.

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POSTER SESSION 3 - P03.053, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The pervasive effects of developmental temperature on the embryos of ectotherms should pose a barrier to range expansion. This challenge is especially great for egg-laying species, which have little capacity to thermoregulate during development. Yet, a great number of oviparous ectotherms occupy broad geographic ranges. Local adaptation in nest site choice could allow parents to provide a suitable developmental environment for their offspring, yet evidence that oviposition site choice influences offspring phenotypes in an adaptive manner is rare. We investigated how geographic variation in the nesting behavior of *Chrysemys picta*, a widely distributed freshwater turtle with temperature-dependent sex determination, influences

offspring phenotypes. We compared predicted offspring sex and survival in maternally-selected nests to a random sample of potential nest sites in six localities across the United States. We found that maternal nesting behavior influences offspring sex; maternally selected sites were more likely to produce female offspring than randomly chosen sites. However, this effect was not always adaptive; in colder localities sex ratios were more biased in maternally selected sites than random sites, potentially indicating a trade-off between optimal sex allocation and offspring survival. Our results suggest that local adaptation in maternal nesting behavior facilitates range expansion in *C. picta*, but that the selective forces driving this behavior vary geographically.



Dynamic Genomes, Dynamic Behaviors: Multi-Omic Mapping of Predator-Induced Gene Regulatory Networks in *Daphnia magna*

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¹KU Leuven

POSTER SESSION 3 - **P03.049**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Behavioural plasticity—the capacity of a single genotype to produce multiple phenotypes in response to environmental cues—is pivotal for survival in rapidly changing ecosystems. Yet, the gene regulatory networks that drive rapid behavioural shifts, the ways in which they vary among genotypes and phenotypes, and the processes by which these networks evolve under natural selection remain largely unresolved. Here, I outline an integrative research project using the freshwater microcrustacean *Daphnia magna*, which exhibits predator-induced shifts in behaviour, to illuminate these questions at three nested levels:

-The Gene Regulatory Network Behind Behavioural plasticity .

How are neural regulatory circuits reconfigured when individuals detect predator cues?

We will employ a combination of single-cell transcriptomic and chromatin-profiling techniques to capture the early and fully developed stages of the inducible antipredator response. By contrasting these molecular snapshots with behavioural assays, we aim to identify key regulatory factors and genes that switch the animal from one behavioural state to another.

- Genotype-dependent network variation.

How does standing genetic diversity shape the architecture and outputs of these networks?

A panel of *Daphnia* clones exhibiting a spectrum of predator-induced behavioural plasticity will be analysed under both control

and predator-cue conditions. Comparative network reconstruction will reveal whether variation among clones arises from differences in upstream regulatory factors, in the wiring of downstream effectors, or in the interactions between them.

- Evolutionary network change.

How do these gene regulatory networks evolve in wild populations facing different predation regimes?

Leveraging a genome-wide association study across natural clones from ponds across Belgium, we will pinpoint genomic regions—and ultimately specific variants—associated with behavioural responsiveness. Additionally, we aim to determine how selection acts on these genomic regions by comparing genotypes of populations with varying fish-predation pressures. Overlaying these findings onto our regulatory maps will clarify whether adaptation preferentially targets regulatory elements, coding regions of network hubs, or multiple loci in concert.

By forging a pipeline from cellular-resolution network dissection, through comparative genotype analysis, to population-genomic inference, this work aims to expose both the core, conserved modules and the flexible, lineage-specific adaptations that underpin predator-induced behavioural plasticity. The resulting candidate regulators, enhancers, and effector genes will lay the groundwork for targeted functional tests (e.g., gene knockdown or editing) and establish a generalizable framework for unraveling the evolution of complex behaviours in ecological contexts.



Evolutionary origin and maintenance of distinct song types that may promote reproductive isolation in the willow tit

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POSTER SESSION 3 - P03.041, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Bird song is a culturally transmitted trait that can drive prezygotic reproductive isolation and speciation in songbirds. The willow tit (*Poecile montanus*) is a resident Palearctic songbird that shows striking geographic variation in song, with three distinct song types occurring mostly parapatrically across its range, separated by either sharp or gradual contact zones. One of these song types – the so-called “Alpine” song, a series of mono-frequency whistles – has a disjunct distribution, occurring in the alpine regions of Europe (Alps, Carpathians, Rhodopes) and in parts of East Asia (China, Kamchatka, Hokkaido), suggesting repeated gains or losses of song types during the evolutionary history of the species.

To gain insight into the evolutionary processes underlying this song type diversity, we sampled 93 individuals from across the willow tit’s distribution range, assembled a chromosome-level reference genome, and performed whole-genome resequencing for all individuals. Phylogenetic and population genomic analyses revealed five major genetic clusters: (1) Chinese, (2) Central Asian, (3) Central European, (4) Japanese (Honshu), and (5) a largely panmictic Northern

Palearctic group. However, these genetic clusters did not correspond to the distribution of song types; rather, the same song types were often shared across genetically distinct populations.

Demographic modeling suggests that all extant genetic groups originated in the early Pleistocene from an ancestral population in China that possessed the “Alpine” song type. As populations expanded northward and subsequently radiated westward and eastward across the Palearctic, a novel song type appears to have been gained, and one of the song types later lost in several isolated peripheral populations (e.g., Central Europe, Britain, Hokkaido, Kamchatka). Species distribution modeling and hindcasting support repeated cycles of range expansion and contraction into subdivided populations throughout the Quaternary, which would enable this scenario while maintaining relatively large effective population sizes.

Our results demonstrate how learned vocal traits can remain evolutionarily stable despite geographic isolation and may contribute to reproductive barriers in contemporary contact zones.



Facing hypoxia/reoxygenation stress in the nervous system of the Mediterranean mussel *Mytilus galloprovincialis*

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POSTER SESSION 3 - **P03.039**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Intertidal organisms have successfully adapted to oxygen (O₂) fluctuations, due to both the intrinsic characteristics of the environment and the deoxygenation of aquatic habitats caused by eutrophication and warming. Facing hypoxia-reoxygenation (H/R) stress requires exquisite homeostatic responses, especially in tissues with a high metabolic demand, such as the nervous system. In hypoxia-sensitive organisms such as humans, neuronal mitochondria are the hub of H/R-induced neural damage, since O₂ deficiency impairs ATP production, whereas an excessive surge of O₂ causes a high production of toxic reactive species and consequently cellular damage. On the other hand, neurons of hypoxia-tolerant species are able to tolerate H/R, taking advantages of molecular tools evolutionarily tested to mitigate the damage associated with H/R.

Here we aim to dissect the cellular and molecular mechanisms underlying the natural resilience by which the neurons of hypoxia-tolerant mussel *M. galloprovincialis* tolerate H and H/R, with a particular focus on mitochondrial homeostasis. We set up a protocol to dissect neuronal ganglia from *M. galloprovincialis* and study them at morphological, cellular and molecular level. The morphology of ganglia has been observed by immunohistochemistry, showing their overall structure and the presence of the different neuronal types. Moreover, we are taking advantages from an experimental system to expose mussels to two different O₂ concentrations: normoxia

(N=7 mg/L O₂) and severe hypoxia (H<0,4 O₂). After 24 hours of H, a first cohort of animals is immediately sacrificed and dissected to obtain the ganglia (H condition). A second cohort is placed into a fully aerated aquarium for 1 hour to induce reoxygenation, prior to tissue isolation (H/R condition). Control animals are maintained in normoxia (N condition). The efficacy of the experimental protocol has been validated by quantifying the expression levels of Hypoxia-Inducible Factor (HIF-1 α) transcript in ganglia and gills (playing a pivotal role in gas exchange). Interestingly, HIF-1 α upregulation during hypoxia is induced at higher levels in gills than in ganglia, suggesting that specific or additional molecular pathways play a role in regulating neuronal adaptation under hypoxic conditions.

We will use molecular and cellular biology techniques, biochemical and computational analyses, imaging, and multi-omic (transcriptomic, miRNomic and proteomic) approaches to identify molecules and molecular pathways allowing neuronal functioning and surviving during extreme O₂ fluctuations. The latter has also a strong biomedical impact: H and H/R occur in medical conditions in humans, such as stroke. Therefore, novel data from hypoxia-tolerant mollusks will bring to light evolutionary adaptations that allow to prevent or mitigate the damage associated with H/R.



Functional adaptation and evolution of mandibular tusks in burrowing mayflies (Insecta: Ephemeroptera) explored via micro-CT and 3D morphometrics

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¹Korea University

POSTER SESSION 3 - **P03.037**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

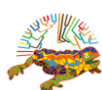
Background: The mayfly superfamily Ephemeroidea, commonly known as burrowing mayflies, is distinguished by the unique morphology and behavior of their mandibular tusks during the larval stage. These tusks resemble those of elephants or mammoths, yet unlike many exaggerated traits found in sexually dimorphic species, they occur in both male and female larvae and are absent in adults. In this study, we investigate the functional morphology and evolutionary trends of these mandibular tusks.

Materials and methods: We examined the functional morphology of mandibular tusks across all six tusked families of Ephemeroidea using 3D morphometrics based on micro-computed tomography, combined with an innovative automatic landmarking method employing point-cloud registration.

Results: Our findings quantitatively support and refine the hypothesis proposed by Bae and McCafferty (1995), demonstrating that mandibular tusk curvature and robustness are functionally linked to burrowing adaptations associated with substrate types in freshwater habitats. Principal component analysis of the mandibular tusks confirmed the differentiation of three dwelling morphotypes: interstitial, burrowing, and hard-substrate burrowing. Our 3D morphometric analysis—encompassing general tusk shape, curvature, center of

mass (CoM), and mass density distribution—further corroborated strong associations between tusk morphology and fossorial behavior. Evolutionary trends inferred from the current Ephemeroidea phylogeny suggest that interstitial dwellers with flattened bodies likely evolved early in the lineages of Potamanthidae and Euthyplociidae. In contrast, cylindrical-bodied burrowers, such as those in the basal lineages of Polymitarcyidae and Ephemeridae, as well as hard-substrate dwellers like Povilla–Ashenopus (Polymitarcyidae) and Cheirogenesia–Palingenia (Palingeniidae), appear to have evolved in parallel—each enhancing burrowing efficiency via modified forelegs. In addition, we report the discovery of a new species, *Drunella koreana* sp. nov., characterized by distinctive cephalic spines and belonging to a separate lineage, identified during our field investigations.

Conclusions: This study applied an integrated 3D morphometric approach to revisit the traditional hypothesis regarding the functional morphology of mandibular tusks in burrowing mayflies. Overall, our findings support the hypothesis proposed by Bae and McCafferty (1995), while extending current understanding of the tusks' functional adaptations. These structures exhibit sophisticated multifunctionality, playing key roles in substrate excavation, filter-feeding, and aggressive interactions.



Genetic and Environmental Drivers of Multiple Cognitive Abilities in Great Tits Living Along an Altitudinal Gradient

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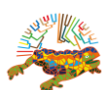
POSTER SESSION 3 - P03.023, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Cognition is a key trait influencing how individuals respond to new environments, and it plays a central role in shaping selection and evolutionary responses. However, the relative contributions of genetic and environmental factors to variation in cognition have rarely been studied in wild populations, leaving a significant gap in our understanding of cognition's role in adaptation to environmental conditions.

To address this, we evaluated variation in cognition in wild great tits (*Parus major*) along an altitudinal gradient and assessed its genetic basis using a combination of quantitative genetic analyses and genome-wide association studies (GWAS). Wild-

caught great tits were tested in temporary captivity to reduce environmental noise and enable standardized cognitive testing. Specifically, we measured associative learning, reversal learning, problem-solving, and detour-reaching performance.

Our results show that cognitive abilities are repeatable, moderately heritable with a few SNPs explaining a percentage of heritable variation. The combination of quantitative genetics and genomic analyses suggests that cognitive traits are at least partially under genetic control. Overall, our study provides evidence that different cognitive abilities have the potential to evolve and points to a polygenic basis for these traits in great tits.



Identification of novel miRNAs in the deep-sea cephalopod *Pteroctopus tetracirrhus* with potential to rescue mitochondrial dysfunction in human diseases.

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POSTER SESSION 3 - P03.040, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

In primary mitochondrial diseases (PMD) associated with severe neurodegenerative and retinal diseases, hypoxic conditions have been shown to partially rescue the phenotype by promoting a metabolic shift from oxidative phosphorylation to glycolysis. Therapies for PMD are limited and either invasive or symptom focused and new therapeutic strategies, such as those RNA-based, are being developed, offering a less distressing approach. Here, we aim to identify novel microRNAs (miRNA) from *Pteroctopus tetracirrhus*, a deep-sea cephalopod species naturally adapted to hypoxic environments, and test their potential to rescue mitochondrial phenotype in an in vitro model of PMD. To discover new miRNAs with potential therapeutic value, we extracted RNA from the retina of 3 individuals and performed small RNA sequencing. The genome of *P. tetracirrhus* is not currently available, thus, the genome of the closely related *Callistoctopus minor* was used for alignments. Mature miRNAs were identified using two independent tools, Prost and miRDeep2. Only the miRNAs detected by both tools in all three biological replicates were retained, resulting in identification of 92 mature miRNAs. Among these, 63 miRNAs showed conservation with known miRNAs in other metazoan species, while 29 were classified as novel. To narrow our focus to species-specific regulators, we selected 18

novel miRNAs that were not conserved with both *Octopus vulgaris* and *Homo sapiens* and had ≥ 10 normalized reads in each of the three samples. These miRNAs, named pte-miR-nov1 through pte-miR-nov18, were used to predict their functional target genes using miRanda and TargetScan, and only the transcripts identified by both algorithms were considered for downstream analysis. Gene Ontology (GO) enrichment analysis was performed on the putative targets of each miRNA to predict their potential biological roles. Four novel miRNAs—pte-miR-nov7, pte-miR-nov8, pte-miR-nov11, and pte-miR-nov14—were identified as strong candidates for rescuing the mitochondrial dysfunction phenotype. Each of them has the potential to target key glycolytic enzymes and genes involved in mitochondrial fusion and fission, or a potential role in modulating inflammation, which is commonly associated with mitochondrial dysfunction. Further analyses will allow us to determine the therapeutic potential of their modulation in a human in vitro model of PMD. Altogether, our work identified novel species-specific miRNAs with potential functional relevance to mitochondrial dysfunction in the hypoxia-adapted cephalopod *P. tetracirrhus* and will address their possible prospective as therapeutics in PMD.



Immunohistochemical mapping of diapause in the aphid brain

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POSTER SESSION 3 - **P03.029**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

All organisms on Earth have evolved biological rhythms as an adaptation to face alternation of periods of favorable and unfavorable environmental conditions, at various temporal scales. Diapause is a state of seasonal dormancy, common in insects and other arthropods, that allows them to overcome recurring periods of adverse environmental conditions. Facultative diapause is triggered by biotic and abiotic factors that precede the arrival of the harsh season. The most prevalent signal that announces the arrival of the inimical season is day length (photoperiod). The capability of many different organisms to detect changes in photoperiod and produce a response is known as photoperiodism. Knowledge on the mechanisms that detect changes in photoperiod, and on the molecular, cellular and endocrine regulatory network that regulates the process in insects is scarce and fragmentary. Different studies have shown the involvement of the circadian clock machinery and of specific clock neurons, as well as of some neurohormones (e.g. insulin-like peptides) and neuropeptides. Aphids are hemimetabolous plant sap sucking insects that have been classically used as models to study photoperiodism. Their life cycles consist of several generations of viviparous parthenogenetic females followed by a single sexual generation, with males and oviparous sexual females. The sexual generation is induced by the shortening of photoperiod after the summer. The outcome of the sexual phase are cold resistant eggs that allow aphids to survive the winter. Thus, induction of the sexual phase is the way aphids enter diapause. Classical studies showed that the aphid brain is the center of photoperiod perception, signal integration

and elaboration of the adequate response that is transduced into the embryos instructing them to develop either as parthenogenetic individuals or as sexual morphs. Development of aphid specific antibodies allowed us to characterize cell groups in the aphid brain along with apparent connections between them, allowing the building of a putative map of the diapause in the aphid brain. These included distinct types of neurosecretory and clock cells located in key brain regions, such as the dorsal and lateral protocerebrum, and also in the lamina. Some of these neurons co-express circadian proteins, such as PERIOD (PER) and the photoreceptor CRYPTOCHROME 1 (CRY1), putatively involved in photoperiod sensing. Their projections toward neuroendocrine centers suggest direct communication between the circadian system and hormonal effectors of diapause. Furthermore, comparison of brains from aphids reared under different photoperiods provided some evidence of the involvement of some of these cells in the process that governs diapause. Together, our findings support the existence of a complex but traceable neuroendocrine network in the aphid brain, where circadian clock neurons, neurosecretory centers, and hormonal outputs are functionally and anatomically connected. We are thus closer to establish a detailed map of the regulatory network that controls diapause in aphids, both at the molecular and cellular levels. Moreover, as the process is likely to be (at least partially) conserved in insects, advances in the completion of this map offers an excellent model to explore the evolutionary adaptations of diapause regulation in other insect species.



Linking Changes in Pollinator Behaviour with Their Effect on Male Fitness in Buzz-Pollinated Flowers

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POSTER SESSION 3 - **P03.052**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

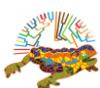
Background: Pollinators play a fundamental role in shaping patterns of pollen transfer that ultimately influence plant fitness. In floral ecology and evolution, pollinators are often treated as a "black box," with limited understanding of how specific behaviours impact reproductive outcomes. In buzz-pollinated systems, bees extract pollen by vibrating flowers, a behaviour that is innate even in naive individuals. After buzzing, bees groom their bodies to collect pollen, storing it in specialized structures (corbiculae) on their hind legs. This groomed pollen is effectively removed from the pool available for pollination, potentially reducing male fitness. In this study, we examine how variation in bee behaviour during floral visits affects pollen dispersal, using *Bombus terrestris* and *Solanum sisymbriifolium* as a model system. By conducting controlled experiments in flight arenas, we aim to provide novel insights into how pollinator behaviour influences pollen fate.

Materials and Methods: To assess pollinator behaviour and pollen transfer, we exposed 5 flowers of *S. sisymbriifolium*, a nectarless, buzz-pollinated plant native to South America, to individual *B. terrestris* bees from captive colonies. Floral visits were recorded in flight cages using a video camera and synchronized audio from a microphone placed inside the cage to record buzzing sounds. We categorized behaviour into distinct actions (e.g., buzzing, grooming on flower, grooming while flying, flying, other) using Behavioural Observation Research Interactive Software (BORIS). Duration and frequency of each behaviour per flower visit were recorded. After each trial, bees were

freeze-killed and their corbicular pollen loads were quantified. The anthers and stigmas from visited flowers were collected to estimate the pollen remaining in the anthers and pollen grains deposited on the stigma.

Results: We analysed behaviours from 52 bees from 10 colonies interacting with 182 flowers. When accounting for total trial duration, bees that allocated a higher proportion of time to buzzing and grooming behaviours were more likely to visit a larger number of flowers. Buzzing and grooming behaviours were positively correlated ($r = 0.83$), especially when grooming occurred on flowers. Grooming while flying was also positively associated with flower visitation rate ($r = 0.68$). Among the behaviours analysed, buzzing ($r = 0.63$) and grooming emerged as key predictors across the different behavioural categories. In contrast, total foraging time was weakly correlated with the different behaviours individually (all $r \approx 0.21-0.35$), suggesting that bees differ not only in how much time they forage but in how they allocate time across specific actions.

Conclusions: This study highlights the ecological importance of how bees allocate time during foraging, not just how long they forage. Behaviours such as buzzing and grooming emerge as key components influencing the outcome of floral visitation. This behavioural investment likely affects pollen transfer dynamics and plant reproductive success, highlighting the role of bee behaviour, which, even at a small scale, can affect the success of plant-pollinator interactions.



Nest weave pattern in weaverbirds: a sexual signal selected through sensory drive?

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POSTER SESSION 3 - **P03.031**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Nests are extended phenotypes that are primarily shaped by natural selection to protect offspring. In some species, however, they are conspicuous and elaborate, and therefore visible to predators. One hypothesis to explain this apparent paradox is that nests could also evolve through sexual selection. While extended phenotypes are increasingly recognized as potential signals, very few studies have tested this theory on a comparative scale. Here, we investigated the potential role of sexual selection in shaping nest visual patterns, focusing on scale-invariance, a property describing how patterns remain visually similar across spatial scales. The sensory drive hypothesis, mediated by a processing bias, predicts that nests whose scale-invariance matches that of natural habitats are processed more easily and appear more attractive. The processing bias hypothesis is new and still lacks empirical validation. Using phylogenetic Bayesian multilevel models in a comparative analysis of weaverbirds (Aves: Ploceidae),

we tested whether their nests have evolved to match the scale-invariance of natural habitats. We quantified the deviation of nest scale-invariance from a range of putative selection optima. We then assessed whether interspecific variation in this deviation is explained by mating system and sexual size dimorphism, two proxies of sexual selection. For both proxies, the estimated parameters had the greatest magnitude for the same putative optimum, supporting a role for sexual selection. Moreover, this optimum aligns with natural habitat values, consistent with the sensory drive hypothesis. Our study provides support for the idea that extended phenotypes, such as nests, may evolve through sensory-driven sexual selection acting on complex pattern geometry. However, our findings highlight the challenge of designing a highly specific test for this hypothesis in the context of visual patterns, and call for further research linking pattern perception and sexual selection.



Non-canonical organisation of the olfactory system in the ant *Temnothorax longispinosus*

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POSTER SESSION 3 - **P03.047**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Communication is essential for group coordination in social animals. In ants, chemical communication is important for regulating division of labour among castes and relies on the detection and processing of chemical cues. Queens and males primarily contribute to reproduction, while workers perform tasks such as brood care, nest defence, and foraging. Odorant receptors (ORs) mediate the conversion of chemical signals into neuronal activity, which is subsequently processed in the brain. This is carried out through a highly conserved olfactory system, where Olfactory Receptor Neurons (ORNs) expressing the same unique OR send projections from the antennae to the same glomerulus in the antennal lobe (AL)—the primary olfactory processing centre in the insect's brain. This spatially precise connectivity constitutes the canonical one-OR:one-ORN:one-glomerulus model of the olfactory system, which usually implies a fixed number of glomeruli in the AL.

Deviations from the canonical one-OR:one-ORN paradigm exist in some species, including ants. In the clonal raider ant (*Ooceraea biroi*), co-expression—more precisely, co-transcription—of OR genes has been observed, particularly among genes belonging to the same tandem array. This different regulation of expression prompts key questions: Do factors relating to worker and colony identity influence OR expression? And does that ultimately determine AL anatomy? In this study, we aim to elucidate the organizational logic of the olfactory system in ants by relating differences in worker brain anatomy and OR gene expression, and their behavioral role in the colony.

Hymenopterans, particularly ants, have undergone an expansion of their OR repertoire, which is hypothesized to facilitate the detection of complex cuticular hydrocarbon profiles and pheromones. In the ant *Temnothorax longispinosus*, workers exhibit age-dependent division of labour; young workers care for the

brood, while older workers primarily forage. However, task allocation is flexible, and workers can switch tasks depending on the requirements of the colony. Our genomic analyses indicate that the *T. longispinosus* genome encodes approximately 460 OR genes, which are arranged in tandem arrays, with many ORs differentially expressed in the antennae of nurses and foragers. We quantified the number of glomeruli in the AL of *T. longispinosus* workers through immunohistochemistry and 3D reconstructions. Six monogynous colonies of different sizes were selected for the experiments. Whole-colony dissections were performed to assess intra-colony variation in glomeruli numbers, enabling the evaluation of individual-level plasticity. Furthermore, inter-colony comparisons were carried out to identify whether AL organization is influenced by factors such as queen genotype, colony size, and environmental factors. Additionally, newly eclosed workers were compared to older workers to assess whether AL organization is fixed post-pupal stage or undergoes plastic changes that are associated with age and division of labor.

According to the one-OR:one-ORN:one-glomerulus model, the number of glomeruli in the AL should correspond to the number of OR genes. However, preliminary results—based on 60 workers from the six colonies—reveal a mismatch between the number of glomeruli and OR genes. Furthermore, differences in glomeruli numbers were observed between newly eclosed ants and older workers, indicating a possible role of developmental plasticity in young adults. Moreover, inter-individual variation in glomeruli numbers was found within workers of the same colony. This variation in the neural architecture may have evolutionary advantages and enhance colony fitness and adaptability. Ultimately, this project will provide new insights into the evolution and plasticity of olfactory systems in insects.



On the evolution of sound-related sexual dimorphism in dung beetles

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POSTER SESSION 3 - **P03.020**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

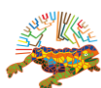
Background: Many aspects of the evolution, ecology, and ethology of dung beetles concern sexual dimorphisms. In many species, the variously shaped horns of males define distinct “maior” and “minor” morphs, whereas they may be smaller or entirely absent in females. Hornless species of dung beetles also exist, and in these sexual dimorphisms are limited to less evident morphological traits (e.g., the presence of spines and teeth on the foretibiae of males, and wider and armless foretibiae in females). Several species of dung beetle are capable of acoustic communication (via stridulation), with relevant intraspecific and interspecific behavioural implications. The aim of the present research was to examine sound-related sexual dimorphism by evaluating if (and how) this trait diverges in horned and hornless species.

Materials and methods: We examined two Palearctic dung beetles: the horned *Copris umbilicatus* (25 individuals from Cantagallo, Bisenzio Valley, N Apennines, Tuscany), and the hornless *Geotrupes mutator* (16 individuals from Mocchie, Susa Valley, W Alps, Piedmont). Although these taxa are not phylogenetically close, they are both large tunnelers, and the adults produce distress calls when disturbed. The stridulatory organs (pectrum and pars stridens) are located on the inner portion of the hind coxa and coxal cavity in *G. mutator* (Geotrupini), and on the distal part of the sternites and elytra in *C. umbilicatus* (Coprini). In accordance with traditional morphometric techniques we used maximum pronotum width as a good proxy of body size. To assess stridulatory organ structure we counted the number of ridges present in a 0.5 mm wide band of the pars stridens. Distress calls in each individual were recorded and the sounds analyzed by testing

the A and B subunits separately (i.e., the sequences of pulses with opposite phases).

Results: Morphometric analysis revealed a clear sexual dimorphism in the stridulatory organ of *G. mutator*, with a significantly higher number of ridges on the pars stridens in females than in males. Sex-dependent differences were also detected in the distress call as the number of peaks in the subunit A was higher and the amplitude of the peaks in the subunit B lower in female specimens. In *C. umbilicatus*, the stridulatory organ showed no evident sexual dimorphism, with males and females presenting approximately the same number of ridges on the pars stridens, and emitting very similar stridulations.

Conclusions: The stridulatory organs (and relative signals) evolved independently and many times in Coleoptera, predation pressure and sexual selection being the main drivers of these morphological innovations. Our results suggest two distinct evolutionary pathways at work. i) *C. umbilicatus* has developed a horn sexual dimorphism but no sexual dimorphism in the stridulatory organs or stridulations emitted, while *G. mutator*, a hornless species, has instead developed a sound-related sexual dimorphism. This may reflect an evolutionary “trade-off” at work: either you invest in the development of one dimorphism, or you invest in the development of another, but not in both. ii) The stridulatory organs of the two species are located in different parts of the body: on the legs in *G. mutator*, on the wings in *C. umbilicatus*. The legs of the males of the hornless *G. mutator* has a tooth that the females lack. Therefore, in this species, the sound-related sexual dimorphism could actually part of the development of sexual dimorphism of the legs.



Patterns and Colors Just Don't Mix: Plumage Color Evolution Trends in a Songbird Clade

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POSTER SESSION 3 - **P03.055**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Feathers are highly specialized structures that display a wide range of colors and patterns commonly used in avian communication, especially in signaling a bird's individual quality to potential mates or rivals. Two complex types of feather coloration are melanin-based patterns (such as spots and stripes) and structural colors (such as blue or glossy black). Both types of coloration require precise coordination of keratin and melanin production not only within each feather, but also across feathers to achieve a uniform appearance in overall plumage. We hypothesized that patterned plumage and structural coloration each signal similar information about a bird's individual quality and are therefore unlikely to co-occur within species. Additionally, we hypothesized that the presence of carotenoid-based coloration such as red, yellow, and orange would have no effect on the evolution of either trait, as these pigments are acquired dietarily and likely signal different types of information. To test these hypotheses, we examined plumage traits across Estrildidae and Viduidae, two closely related passerine families with highly diverse plumage colors and patterns. We

scored for the presence or absence of melanin-based patterning, structural coloration, and carotenoid coloration from over 200 museum specimens across both families. We reconstructed ancestral states for each trait and used phylogenetic mixed modeling to test for correlated evolution of color traits. Consistent with our hypothesis, we found a negative association between patterned plumage and structural coloration in extant species. Additionally, habitat type and presence of carotenoid coloration significantly predicted the evolution of structural coloration, but not patterned plumage. Finally, a test for correlated evolution using Pagel's method revealed that the likelihood of either patterned or structural plumage evolving depends strongly on the state of the other trait. Our results suggest that patterning and structural coloration are redundant signals in Estrildidae and Viduidae and that this trend may be present in other avian groups, though future studies are needed to test this. To our knowledge, this study is the first to examine the relationship between structural color and plumage patterns across a group of closely related birds.



Personality development in wild house mice: Evidence for a nutrition-dependent sensitive period early in life

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*POSTER SESSION 1 - **P01.015**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM*

Changing environmental conditions pose challenges to organisms, for example, by disrupting access to food. Across species and traits, animals use phenotypic plasticity to adjust to such changes. Previous work has demonstrated that wild house mice are able to adjust stress coping to changing food quality within just three generations. However, we do not know when during ontogeny changing conditions induce phenotypic adjustments. We tested experimentally when during ontogeny (as fetus, newborn, weanling, or late adolescent) a food switch between standard and high-quality food shapes personality development (stress coping and stress perception) in cage-housed, wild house mice (*Mus musculus domesticus*). Personality

traits were assessed in the Open Field and the Elevated Plus Maze at different time points during ontogeny (weaning, early adolescence, late adolescence, and adulthood). We highlight two key findings. First, the fetal life stage might be a sensitive period for stress coping in response to experiencing decreases in nutritional quality. Second, experiencing an increase in nutritional quality may slow the age-related switch towards a passive stress-coping strategy. Our study contributes towards understanding the complex relationships between development, nutrition, and personality. These insights can inform future studies investigating how nutritional fluctuations shape individual behavior and population dynamics in the wild.



Rapid color change in cichlid fishes: Using computer vision to study behavioral diversity and evolution

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POSTER SESSION 3 - **P03.027**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

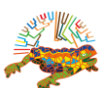
Background: The ability of some animals to rapidly change colors, sometimes within seconds, is a fascinating adaptation to interact with the biotic and abiotic environment. In cichlids, a very diverse and species-rich fish family, this ability serves as a powerful mode of social communication. By fading and enhancing dark (melanic) markings on the face and body, these fishes can signal sexual interest to potential mates or aggression towards competitors. In addition, cichlids may change some of the same markings to achieve background matching or the motion dazzle effect, which can help them avoid detection and tracking by others, including potential predators or prey. However, due to the challenge of quantifying color change behavior, it is poorly understood how color change ability and behavior differ between cichlid species and individuals, as well as how they are linked to different environmental stimuli and factors. Overcoming this barrier will help shine a light on many aspects of the behavioral ecology of cichlids and the evolution of behavioral traits.

Materials and methods: Here, we developed a computer vision pipeline to quantify the state of different melanic markings of cichlid fish in high temporal resolution under laboratory conditions. We show that rapid color change can be elicited by manipulating the environmental factors cichlids use as cues, such as ambient light intensity and background brightness. To deliver stimuli to fish in small aquaria with consistency and precision, we developed purpose-built experimental setups that use single-board computers to coordinate both stimulus

delivery and data acquisition. The current dataset encompasses such recordings for multiple cichlid species with individuals differing in sex, age, reproductive states, and color morph and will provide a solid foundation to expand these analyses to freely behaving animals in social contexts.

Results: The data collected proves that it is possible to use computer vision to characterize rapid color change responses to ecologically relevant environmental stimuli and identify general trends in how different markings respond, as well as compare such responses between species and individuals. Our preliminary findings include a thorough investigation of how the same marking responds in different color morphs of a species, as well as multiple types of responses to the same environmental stimuli. Our data supports consistent responses within and between individuals and provides evidence for interactions between specific markings, environmental stimuli and social status.

Conclusions: Investigating the evolution of behavior remains one of the biggest challenges in biology. Studying behaviors via rapid color change, where behavioral and motivational states of an animal can be extracted from video frames, holds significant potential to link behavior, genetics, and environmental context. Here, we showcase a computer vision pipeline that can acquire a direct phenotypic (behavioral) read-out in a highly automated manner that will be useful for investigating environmental stimuli such as light and background, as well as social stimuli.



Role of niche choice in rapid host adaptation of a global pest

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POSTER SESSION 3 - **P03.035**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The niche choice mechanism, which describes the process of organisms choosing different microenvironments matching their phenotype, affects individual survival and reproductive success. It can influence many ecological and evolutionary processes by promoting rapid local adaptation and maintaining genetic diversity. The evolution of niche choice and its role in individual adaptation remain largely unknown and the question of how niche choice and its evolution affect insecticide resistance in phytophagous insects is yet to be addressed. Using experimental evolution, metagenomics and microbiome analyses, this study aims to investigate how oviposition choice affects resistance evolution and overall fitness in Colorado Potato Beetles (CPB), a notorious global pest.

We evolved beetle populations for at least 12 generations in separate cages in a controlled greenhouse environment. A 2x2 factorial design was implemented: presence or absence of plant treatment variation (niche difference, ND+/ND-) and presence or absence of oviposition choice (OC+/OC-). In ND+ treatments, beetles could choose between insecticide-treated and water-treated potato plants; in ND- both trays were of identical plant quality. In OC+, larvae were placed at their maternal oviposition site, whereas they were placed in random distribution in the OC- treatment to disregard the female's choice. Fitness, oviposition behavior and survival were recorded in each generation. Additional bioassays were performed to test oviposition preference and insecticide resistance. These bioassays were performed on offspring beetles that were reared on untreated plants to identify possible genetic mechanisms behind this behavior and exclude maternal effects. So far, we performed the choice assay for generation F1 and the toxicity assay for F1 and F4.

The choice bioassay revealed a trend toward oviposition preference on water-

treated plants by F1 females whose parents (F0) experienced the oviposition choice treatment (OC+). The choice index for this group indicated avoidance of insecticide-treated hosts, while females from the other three treatment groups showed no such pattern. This trend narrowly missed statistical significance ($p = 0.0546$). Feeding preference showed no significant differences between treatments ($p = 0.6$). Preliminary toxicity assay results indicated increased resistance to imidacloprid in F4 populations compared to the founder generation across all treatments. The strongest increase in LC50 values was observed in the insecticide-only group, followed by the “no choice” (OC-) and “oviposition choice” (OC+) treatments and lastly the water-only treatment. These findings support the hypothesis that the presence of niche choice behavior may slow the evolution of insecticide resistance.

For bioassays, preliminary findings showed a trend toward avoidance of insecticide-treated hosts in the F1 populations of OC+ treatment. Since the larvae for bioassays were reared exclusively on untreated plants to avoid maternal imprinting effects, this avoidance behavior might be genetic or result from random variation. The insecticide resistance increased across all treatments by generation F4, most prominently in the insecticide-only treatment. These results may indicate a potential trade-off between behavioral avoidance and physiological resistance: beetles that evolved under oviposition choice conditions may rely more on insecticide-treated host plant avoidance, thereby reducing selection pressure for physiological resistance. In contrast, beetles lacking the choice behavior may be more exposed to insecticides, promoting faster evolution of resistance mechanisms. Further long-term data- involving microbiome analyses and genomic profiling- is currently gathered to test this hypothesis.



Sex differences in parental cognition and response to offspring signals in birds

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POSTER SESSION 3 - P03.036, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Mothers, fathers, and offspring clash over how much food offspring should get. Both fathers and mothers benefit from having well-fed offspring, but each would prefer their partner shouldering more, ideally all, of the burden of care, leading to sexual conflict over offspring provisioning. Different selective pressures acting on males and on females may have caused the evolution of sex differences in how parents respond to offspring begging signals when making provisioning decisions. However, despite knowing much about what choices parents make overall, we know less about broad patterns of sex differences in parental response to begging or about the neurobiological and cognitive basis underlying parental choices. Here, we combined an experimental study on wild great tit parents (*Parus major*) to explore sex differences in how quickly and confidently parents choose an offspring to feed, with a comparative study across 31 bird species to explore the evolutionary drivers of sex differences in parent-offspring communication. In our experimental study, we found that great tit parents' decision speed was impacted by offspring starvation

risk, decision complexity and whether parents chose to feed a preferred chick or not, in a sex-dependent manner. Specifically, females make slower decisions than males and moved food between chicks more before committing. In our meta-analysis, we found that species varied in whether males or females were more responsive to offspring begging, and that this is correlated with pair bond strength. In species with weaker pair bonds, such as the great tit, females are more responsive to offspring begging. Conversely, males are more responsive to begging in species with stronger pair bonds, such as the barn owl which normally mates for life. Together, these results suggest that variation in sexual conflict may affect the evolution of within-species variation in parent-offspring communication, and that this variation may be caused by differences in how males and females integrate sensory information and reach a decision threshold. More generally, they show the benefits of combining multiple approaches when exploring the causes of behavioral diversity.



Sex-specific voluntary fasting in the ontogeny of size dimorphism in snakes

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POSTER SESSION 3 - **P03.048**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Variation in energy intake is often viewed as a response to ecological constraints or food availability, with sex-specific reproductive investment driving divergent growth and the evolution of sexual size dimorphism. But what if causality runs in the opposite direction? Could intrinsic, sex-specific growth trajectories shape feeding behaviour instead? We tested this idea in a four-year laboratory study of the sand boa (*Eryx colubrinus*), a strongly female-larger species, under continuous food availability. Growth, feeding, and body condition were tracked over time, and growth was experimentally manipulated via exogenous testosterone. Our results reveal that growth in body length proceeded independently of immediate food intake. Instead, feeding and fasting were tightly regulated by body condition: snakes stopped feeding upon reaching an upper condition threshold, and resumed only after condition declined to a lower limit. These thresholds were shared across sexes and hormone treatments. Males fasted more often and for longer,

months lasting periods than females. Faster-growing females depleted energy reserves faster, which led to more regular feeding. Testosterone had no effect on growth or feeding in males, but slowed (defeminized) growth and reduced feeding frequency in females—supporting the idea that feeding is driven by growth-mediated shifts in body condition. These findings challenge classical energy-budget models. Instead, they support a model where feeding serves to maintain body condition within narrow limits, and growth rate determines how quickly those limits are reached. Extended fasting in males may be an adaptive strategy to reduce the risks associated with feeding in snakes—such as predation or injury—while female growth shaped by fecundity selection necessitates more frequent feeding. This study highlights the need to integrate behaviour, morphology, and physiology to understand the evolution of sexual size dimorphism and sex-specific life-history strategies.



Testing the Darwin-Bateman Paradigm: Anisogamy as a Fundamental Driver of Sex Role Evolution in Birds

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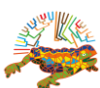
POSTER SESSION 3 - P03.017, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The evolution of sex roles—mating systems, parental care, and sexual size dimorphism—has been a central question in evolutionary biology since Darwin. Over the past 50 years, multiple hypotheses have been proposed to explain sex role variation, including sexual selection, natural selection via niche partitioning, and demographic factors such as sex-specific mortality and adult sex ratios. However, despite extensive research, the foundational hypothesis of sex role evolution—the role of anisogamy—has never been rigorously tested across taxa.

The Darwin-Bateman paradigm posits that anisogamy, the inherent disparity in gamete size between sexes, drives fundamental differences in reproductive investment, competition, and parental care. To empirically test this long-standing theory, we assembled the most comprehensive dataset to date on male and female gamete sizes in nearly 200 bird species, a group with unparalleled data on mating systems and parental strategies. Using cutting-edge phylogenetic comparative methods, we

modeled the relationship between anisogamy (gamete size bias) and multiple sex role components. Additionally, we conducted phylogenetic path analysis to assess the relative explanatory power of anisogamy compared to alternative hypotheses, including demographic constraints and ecological niche partitioning.

Our preliminary results reveal significant patterns, suggesting that anisogamy may play a more central role in shaping sex roles than previously recognized. At ESEB 2025, we will present the full results, offering the first large-scale empirical test of a fundamental yet unverified evolutionary theory. By integrating detailed empirical data with advanced phylogenetic modeling, this study provides a novel framework for understanding sex role evolution. While our findings are grounded in birds, they hold broad implications for the evolution of reproductive strategies across the tree of life.



The effects of dietary innovation on metabolism during the evolution of cognitive enhancement in *Heliconius* butterflies.

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POSTER SESSION 3 - **P03.026**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The colonisation of a new dietary or ecological niche can drive sensory and cognitive adaptation, but the production and maintenance of neural tissue comes at a significant energetic cost. Understanding how selection balances the costs and benefits of neural investment is a key issue in evolutionary biology. Such investment may be offset by reductions in other metabolically demanding tissues or processes, with consequences for life history traits and behaviour. Alternatively, behavioural innovations that increase energy intake may relax these constraints, permitting greater investment in energetically costly brain regions. Among butterflies, *Heliconius* are unique in that they consume both nectar and pollen as adults. This dietary innovation provides a consistent and protein-rich resource that supports their extended lifespan and is associated with improved cognitive performance and the expansion of mushroom bodies – brain structures implicated in learning and memory. These elaborated brain regions are likely to impose increased metabolic demands. It is hypothesised that the shift to pollen feeding in *Heliconius* alleviated the energetic constraint typically limiting neural expansion, enabling increased investment in cognitive architecture. This project aims to test this hypothesis by comparing metabolic investment in *Heliconius* to that of non-pollen-feeding *Heliconiini* at multiple levels.

At the whole-organism level, resting metabolic rates are being estimated via flow-through respirometry, using custom-designed chambers and infrared video tracking to identify periods of rest. Trial experiments have successfully demonstrated the feasibility of this approach, and data collection is currently ongoing across a wide phylogenetic sample. To complement organismal-level measures, oxygen consumption rates of dissected whole brains are being quantified using high-throughput microrespirometry. While early trials revealed challenges due to the size and respiration rate of butterfly brain tissue, the protocol has been refined to focus on basal respiration. Once final adjustments are made, this will enable efficient comparative analysis across multiple species. Finally, activity of cytochrome oxidase, a metabolic enzyme and proxy for ATP usage, will be measured in whole brains and mushroom bodies using histochemical staining to assess regional differences in energy metabolism. Together, these approaches will help elucidate whether dietary shifts such as pollen feeding allow for increased energy allocation to neural tissue, and to what extent cognitive elaboration is constrained or enabled by energetic factors. These data will contribute to a broader understanding of how ecological innovation can influence brain evolution and behavioural complexity.



The effects of migration-related genotype on swimming behavior in rainbow trout *Oncorhynchus mykiss*

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POSTER SESSION 3 - **P03.042**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Migration occurs across a wide range of taxa, with each group exhibiting several distinct strategies. Salmonid fishes are a prime example of diversity in migratory behavior, as differences in migration strategies are observed not only between species, but also between ecotypes of the same species. While some salmonids are residents (i.e., non-migrating), the life cycle of anadromous (i.e., migrating) salmonids typically consists of migrating from freshwater, where they hatched, to the sea, where they feed and grow, and then back to freshwater to spawn, often in their natal river. In anadromous *Oncorhynchus mykiss* (steelhead), spawning migration timing is a variable trait, as two separate runs are observed: one in summer and one in winter. Both ecotypes spawn in the spring, indicating strong differences in the energetic costs of spawning migration, as the summer-migrants reside in the river for several months before spawning. The *greb1L* locus on chromosome 28 has been associated with variation in spawning migration timing in *O. mykiss*, yet the physiological and behavioral processes underlying this link remain unknown. Thus, disentangling the roles of genetic, physiological and environmental factors governing migratory behavior, as well as their interactions, is key to

understanding the evolution and diversity of migratory strategies in salmonids. In this study, 144 immature females from an aquaculture strain of rainbow trout (originating from a California Steelhead population) were reared in common garden conditions and implanted with accelerometry loggers to measure routine activity. Individuals were homozygous either for the summer- or for the winter-run *greb1L* allele. We hypothesized that the summer-run allele of *greb1L* would be associated with a “fat-storing” phenotype, characterized by reduced activity and increased fat deposition to conserve energy for a longer spawning migration. A food-restriction treatment was also implemented to test whether behavior is modulated by food availability in interaction with *greb1L* genotype. Activity was measured for 24 consecutive hours every six days from January to May 2025. Growth in body mass and length, and the total amount of fat reserves—quantified via Dual-energy X-ray absorptiometry (DEXA) imaging—were also assessed to evaluate differences in energy allocation to somatic growth and fat deposits. The results provide insights into the genetic bases of phenotypic characteristics associated with alternative migratory behaviors in a key migratory fish species.



The evolution of socially-mediated dispersal favours the emergence and maintenance of distinct social and dispersal strategies

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POSTER SESSION 3 - **P03.050**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Kin selection is an important mechanism for the evolution of cooperative behaviours across multiple taxa. While limited dispersal can foster kin selection by generating a genetic correlation between cooperating individuals, it also increases competition among relatives, constraining the evolution of cooperation. Prior theory has explored the co-evolution of dispersal and cooperation but typically assumes dispersal is independent of social cues. Here, we use mathematical modelling to examine whether socially-mediated dispersal, where individuals adjust their dispersal based on social context, can mitigate kin competition and thus enhance cooperation. We model the joint evolution of: (i) the probability of cooperating within social groups; and (ii) the probability of dispersing conditional on the number of individuals that have cooperated within the group, leading to a reaction norm

for dispersal. We show that when the probability of dispersal increases with the number of cooperators, cooperation is favoured due because it increases the fitness of relatives. The joint evolution of the two traits can lead to the differentiation of two types of individuals, one that always cooperates and another that never does. Although both types evolve dispersal norms such that they disperse more often when there are more cooperators in the group, cooperators evolve a steeper norm, reflecting greater sensitivity to their social environment. Our study shows that dispersal norms in response to an individual's social environment can depend on that individual's own social tendency, which can help explain how dispersal proclivities may differ between individuals and between environments within a single population.



The role of social learning in speciation by sexual selection: the case of a poison frog

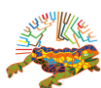
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POSTER SESSION 3 - **P03.028**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

After 150 years of research, the topic of speciation by sexual selection remains a frustrating endeavor, in which, empirical evidence fails to meet theoretical predictions. An outstanding progress was recently achieved by showing that sexual learning at early stages of development (imprinting), a phenomenon that occurs during parental care, facilitates mating traits divergence and the preferences for them, the initial steps of reproductive isolation. Imprinting leads females to mate with males that look like their parents (and usually themselves), a process that hinders gene flow between divergent phenotypes. Imprinting also leads males to compete against individuals that look like themselves, relaxing competition pressures and promoting co-existence among divergent phenotypes. Much less understood is the

role that social learning at other life stages and by different tutors - a common process in animals - plays in the facilitation of behavioral reproductive barriers. To fill this gap, I performed female mate choice and male territorial aggression experiments in both field conditions and a captive bred colony of the poison frog *Oophaga pumilio*, a species that shows rapid and dramatic divergence in a mating trait (color). I will show results of two experiments that suggest that individuals' sexual biases are modified by their social environment (i.e., the phenotypes that they interact with) both during development and after sexual maturity. This suggests that certain social environments can rapidly trigger speciation, providing a unique path towards the generation of biodiversity in our planet.



To migrate or not to migrate? Exploring the genomic basis of the partial migratory behavior in bats

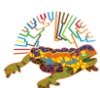
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POSTER SESSION 3 - P03.021, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Migration is a widespread phenomenon across the animal kingdom, involving the seasonal, long-distance movement of individuals in search of favorable environmental conditions. This complex behavior is supported by a suite of morphological, physiological, and behavioral adaptations, and while it is often environmentally triggered, increasing evidence suggests that it is also genetically regulated. Partial migration, where migratory and resident individuals coexist within the same population, is thought to represent an evolutionary intermediate towards obligate migration, shaped by opposing selective pressures. However, despite decades of research on migratory ecology and orientation, the genetic and molecular underpinnings of migration remain poorly understood, especially in mammals. Here, we investigated the genomic basis of migration in a flying mammal, the lesser long-nosed bat (*Leptonycteris yerbabuenae*), a partially migratory, nectar-feeding species that maintains tight ecological interactions with columnar cacti and agaves. In this species, one group of female mates in southwest-center Mexico (October–December) and migrates northward in spring to give birth in the Sonoran Desert. A second group remains resident in central Mexico, mating and giving birth during the summer. All adult males are also resident in central Mexico and mate with both groups of females. Using RAD-seq, we found no evidence of neutral genetic structure across these populations, consistent with high gene flow and panmixia, despite their opposite migratory movements.

Both migratory and resident groups showed similar demographic histories, with an initial period of stability followed by a decline starting ~30,000 years ago during the Last Glacial Maximum. This period coincides with a shift in the distribution of their main food plants and the emergence of migratory behavior. Despite the lack of genetic structure, we identified 23 genomic regions potentially involved in determining migratory behavior. These regions were detected using an iterative and complementary approach based on F_{ST} and Tajima's D , focusing on candidate loci showing signatures of selective sweeps (e.g. F_{ST} values several orders of magnitude above the genome-wide average). Ten of these regions also showed signatures of positive selection in the migratory group. The annotation showed some of the candidate regions contained genes related to nervous system and immune function, some of which have been previously implicated in migratory behavior in other vertebrates, suggesting functional convergence. Our findings support the hypothesis that migration in *L. yerbabuenae* has a genetic basis shaped by strong diversifying selection acting on specific genomic regions. The stable coexistence of migratory and resident females within a panmictic population may reflect a form of balancing or disruptive selection operating under a “migratory threshold” model. In this scenario, males could influence phenotypic transitions across generations through their homogenizing genetic contribution. Overall, this study highlights the potential of bats as valuable models for understanding the evolutionary origins and genomic underpinnings of migratory behavior.



Transcriptomic mapping of diapause in the aphid brain

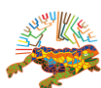
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POSTER SESSION 3 - **P03.030**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

All organisms on Earth have evolved biological rhythms as an adaptation to face alternation of periods of favorable and unfavorable environmental conditions, at various temporal scales. Diapause is a state of seasonal dormancy, common in insects and other arthropods, that allows them to overcome recurring periods of adverse environmental conditions. Facultative diapause is triggered by biotic and abiotic factors that precede the arrival of the harsh season. The most prevalent signal that announces the arrival of the inimical season is day length (photoperiod). The capability of many different organisms to detect changes in photoperiod and produce a response is known as photoperiodism. Perception of changes in photoperiod triggers a, yet only partially characterized, cascade of molecular, neurologic and endocrine events that lead to the activation of diapause in insects. Different studies have shown the involvement of the circadian clock machinery and of specific clock neurons, as well as of some neurohormones (e.g. insulin-like peptides) and neuropeptides. Aphids are hemimetabolous plant sap sucking insects that have been classically used as models to study photoperiodism. Their life cycles consist of several generations of viviparous parthenogenetic females followed by a single sexual generation, with males and oviparous sexual females. The sexual generation is induced by the shortening of photoperiod after the summer. The outcome of the sexual phase are cold resistant eggs that allow aphids to survive the winter. Thus, induction of the sexual phase is the way aphids enter diapause. Classical studies

showed that the aphid brain is the center of photoperiod perception, signal integration and elaboration of the adequate response. Previous research from our group has identified putative elements of the regulatory network involved in aphid diapause at the levels of perception, integration and signal transduction. Aiming at filling the gaps in the signalling pathway of aphid diapause we compared expression between aphid brains in Long Day condition (promoting parthenogenesis) and aphids in Short Day condition (inducing sexual morphs) by two complementary approaches: 1) scRNAseq of aphid brains and 2) in toto RNAseq time course of aphid brains. The scRNAseq produced the first atlas of an aphid brain and describes different cell groups. Regarding the regulation of diapause, we found genes coexpressed in the Insulin Producing Cells, Dorsal Neurons and Lateral Neurons, pointing out the signalling pathways that may be involved in diapause regulation. The in toto brain RNAseq time course contributed to the identification of circadian regulated genes and provided candidate pathways to regulate diapause. These results complement with the localization by Immunohistochemistry of different neuropeptides and proteins in the aphid brain. Altogether, we are closer to establish a detailed map of the regulatory network that controls diapause in aphids, both at the molecular and cellular levels. Moreover, as the process is likely to be (at least partially) conserved in insects, advances in the completion of this map will help to map diapause in other insect species.



Warmer climate disrupts pace-of-life syndromes through ontogenic changes of activity levels in the common lizard *Zootoca vivipara*

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POSTER SESSION 3 - **P03.025**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Ongoing climate change has drastic and diverse effects on organisms. In particular, warmer temperatures are assumed to accelerate the pace of life of individuals, and therefore developmental and senescence rate. Because behavioural traits and their among-individual variability are involved in eco-evolutionary dynamics, a better understanding of how climate is susceptible to alter their ontogeny is required. Here we focus on the effect of a warmer climate on the ontogeny in individual activity level in the common lizard *Zootoca vivipara*. Activity is more susceptible to changes in climate given its link with pace of life syndrome and metabolic rates and such changes may have strong consequences on population and community dynamics. For the last 10 years, common lizards are maintained in semi-natural mesocosms experiencing either current or ~2°C warmer climatic conditions and individually monitored for activity level and life history traits over their lifetime. Using this database, we explored how warmer climates impact age-related changes in the mean and the within and between individuals variability of activity levels as well as the mechanisms potentially involved in such

changes (differential survival and plasticity). We found activity to be partly heritable and repeatable only on parts of the lifetime resulting from contrasting patterns of between and within individuals variability across ages and climates. We found that activity level decreased with age, both as a result of within-individual changes and of the selective disappearance of more active individuals, more pronounced in the warmer climate. Overall, the syndrome linking activity level and fitness outcomes was climate-dependent, mostly because only less active individuals accelerated their pace-of-life in warmer climates. Considering its close links with physiology and pace of life syndromes, observed changes of the ontogeny of activity with climate may have consequences on the population dynamics and persistence of this generalist predator species with potential cascading effects on species at lower trophic levels and whole ecological networks .

Key words: climate change, activity, common lizards, ectotherms, between-individual variability, repeatability, heritability, animal personality



Chemosensory evolution at the origin of inquilinism in the bee louse fly (*Braula coeca*)

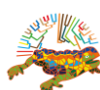
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MEETING ROOM 115, August 18, 2025, 10:30 AM - 12:30 PM

Living beings are not isolated and interact with each other. These interactions are often mediated by chemical odorants but how the evolution of the olfactory system can determine intra- and inter-specific interactions remains unclear. A peculiar example is the queen mandibular pheromone (QMP) in *Apis mellifera*, which inhibits the development of the ovaries in the conspecific workers (hence leading to social cohesion) and also in other insect species (hence protecting the colony). Of the different parasites of *Apis*, only one, the blind and wingless bee louse (*Braula coeca*), paradoxically spends its entire life cycle as an inquiline in the colony, clinging to the thorax or head of bees, with a clear preference for the queen at the end of the summer. We have sequenced its genome and confirmed that it is a basal branch of the *Drosophilidae* family, closely related to *Drosophila melanogaster*. To understand how *Braula coeca* has found an evolutionary

means of circumventing the anti-ovarian effect of QMP, we investigated the evolution of multiple chemosensory gene families. We found the bee louse has lost almost half of the olfactory receptor drosophilid repertoire, including some that respond to QMPs in *D. melanogaster*. By combining multiple functional approaches, including behavioral assays in the field, electroantennography, heterologous gene expression of *Braula* receptors in *D. melanogaster* empty neurons, and CRISPR-Cas9 genome editing to induce *B. coeca* mutations in *D. melanogaster*, we are currently testing the hypothesis that the rapid evolution of *Braula coeca*'s ORs has protected it from the anti-ovarian effects of QMP, enabling it to reproduce in an essentially sterilizing environment. Primary results are supporting this hypothesis, hence providing significant insights on how changes in chemosensory genes promote the evolution of inter-specific interactions.



Convergent evolution of pain insensitivity in a new *Heliophobius* species

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MEETING ROOM 115, August 18, 2025, 10:30 AM - 12:30 PM

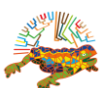
The African mole-rat family, Bathyergidae, is a family of subterranean rodents that exhibit extraordinary physiological adaptations to life underground. Among these, variation in pain sensitivity is particularly striking (Eigenbrod et al., 2019). We have discovered a population of *Heliophobius* mole-rats from Tanzania's Usambara Mountains that are uniquely insensitive to both acid and capsaicin, two compounds that typically induce robust pain behaviors (algogens) when injected into the animals' hindpaw. Until now, this exact phenotype was only reported in the naked mole-rat (*Heterocephalus glaber*; Park et al., 2008; Eigenbrod et al., 2019). The newly identified *Heliophobius* population, like *H. glaber*, shows no nocifensive response to these stimuli. Interestingly, however, they remain sensitive to AITC (allyl isothiocyanate), a pungent compound that activates the TRPA1 ion channel, indicating that some pain pathways remain functional. In contrast, their closest known relative, Emin's mole-rat (*H. emini*), shows normal pain responses to all three algogens, similar to the mouse and reflecting the typical mammalian nociceptive phenotype. This stark interspecific contrast provides a powerful natural experiment to investigate the evolution and molecular basis of nociceptive variation.

To elucidate the genetic mechanisms underlying this difference, we generated high-quality, chromosome-level genome assemblies for both *Heliophobius* taxa using PacBio HiFi long-read sequencing and Hi-C chromatin contact mapping. We also generated genome assemblies for other Bathyergid species to enable broader comparative analysis. Using an updated naked mole-rat reference genome for alignment and annotation, we identified two amino acid substitutions in the

SCN9A gene (encoding the voltage-gated sodium channel Nav1.7) in the Usambara *Heliophobius*. These substitutions, located in complementary domains of the pore region of the channel, are identical to those previously shown to underlie acid insensitivity in *H. glaber*. Notably, these substitutions are absent in *H. emini*, strongly suggesting a case of convergent molecular evolution leading to shared nociceptive phenotypes.

In addition to functional genomics, we conducted phylogenomic analyses to clarify the evolutionary relationships of this newly identified population. Genome-wide markers generated via ddRAD sequencing, along with mitochondrial cytb sequences, place the Usambara mole-rat in a distinct clade, separate from both *H. emini* and *H. argenteocinereus*. Our sample from this population clusters with individuals previously assigned to *H. kapiti* (Uhrova et al., 2022), a taxon of uncertain status. Taken together, the genomic, phenotypic, and phylogenetic evidence supports recognizing *Heliophobius kapiti* as a distinct species.

This newly recognized species expands our understanding of African mole-rat diversity and provides a unique model for studying convergent evolution in sensory biology. The geographic and phylogenetic proximity of this taxon to the naked mole-rat (*H. glaber*)—despite their belonging to different genera—makes the Usambara mole-rat an exceptional system for investigating how similar selective pressures can independently shape complex traits such as pain insensitivity. Our findings demonstrate that extreme sensory phenotypes can evolve multiple times within the same family and offer a window into the genomic architecture that enables such adaptations in mammals.



Ecological divergence and post-eclosion brain development shape visual performance in *Heliconius* butterflies

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MEETING ROOM 115, August 18, 2025, 2:00 PM - 5:00 PM

Linking ecological divergence to neural and behavioural variation is central to understanding the evolution of behavioural diversity. In the closely related butterflies *Heliconius cydno* and *H. melpomene*, ecological speciation across forest light environments is associated with pronounced differences in visual system morphology. *H. cydno*, which inhabits darker, closed-canopy forests, exhibits larger eyes and greater neural investment in vision-associated brain regions than *H. melpomene*, a species found along brighter forest edges. However, the behavioural and developmental implications of these visual system adaptations remain poorly understood. Using an optomotor response paradigm, we assessed visual acuity in both species across four stages following adult emergence. We found that visual acuity increases with age in both species, with *H.*

cydno consistently exhibiting higher resolution. These ontogenetic improvements in visual performance coincide with the onset of key life-history behaviours, such as male courtship and female oviposition, suggesting that early adult neurodevelopment may facilitate behavioural maturation. To explore the neural basis of these patterns, we quantified brain development using confocal microscopy and a novel deep-learning segmentation pipeline to analyse over 80 brain samples. Our analyses revealed significant species differences in optic lobe volume that align with the enhanced visual acuity observed in *H. cydno*. By integrating ecological context, neuroanatomy, and behaviour, our findings demonstrate how environmental pressures may drive neural specialization and shape behavioural trajectories early in the speciation process.



Evolution of Taste Neurons and Behaviour

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MEETING ROOM 115, August 18, 2025, 10:30 AM - 12:30 PM

How sensory perception evolves as species diversify and change ecological specialization is poorly understood. In chemosensation, historically more attention has been placed on olfaction, leaving open important questions about how taste and contact chemosensing evolve and the behavioural differences that they drive. We have carried out a comparative single-cell transcriptomic (snRNA-seq) study of neurons in the taste appendages (forelegs and proboscis) of closely-related *Drosophila* species with distinct ecologies (*D. melanogaster*, *D. simulans*, and *D. sechellia*). Analyses of the neuron transcriptomes revealed unambiguous homologous cell-types. Among the most pronounced changes were lineage-specific switches in sex-biased gene expression. These findings bolster recent observations made at the bulk tissue level but narrow them to specific neuron populations and point towards functional nervous system differences evolving between the sexes. Cross-species analysis revealed the most quickly evolving neurons in the forelegs are chordotonal neurons (vibration sensors) followed by mechanosensory and gustatory

neurons. In the proboscis, the most rapidly evolving neurons are a population of uncharacterised neurons that express nicotinic acetylcholine receptors. To investigate the functional consequences of these gene expression changes in specific neuron populations, we focused on a striking species difference involving a putative pheromone receptor belonging to the pickpocket channel family, *ppk15*. In *D. melanogaster* and *D. simulans*, *ppk15* is expressed in leg and proboscis neurons. In *D. sechellia*, the proboscis expression of *ppk15* has been lost. Using a panel of transgenic reagents and *ppk15* null mutants generated for the three species, we are using calcium imaging to measure physiological responses and characterising feeding and mating phenotypes. Our results point towards *ppk15* being a conspecific pheromone receptor that's expression has been recently lost specifically from the proboscis of *D. sechellia*. Together, our analyses underscore the utility of combining global and single gene studies to advance our understanding of how taste and taste-related behaviours evolve over short evolutionary timescales.



Genetics and environment shape vocal frequency variation in birds

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MEETING ROOM 115, August 18, 2025, 2:00 PM - 5:00 PM

Vocal frequency in animals is shaped by both intrinsic and extrinsic factors. A role of the environment in driving acoustic adaption is widely supported. Climate may also shape frequency both directly, via physiological effects on the vocal tract, but also indirectly through morphological adaptation to climate and temperature-dependent physiological constraints on song frequency. But much variation in vocal frequency has evolved over time between related species that cannot be attributed to environmental effects. Determining the relative effects of genetic and environmental factors and their interactions on vocal frequencies is a complex undertaking hitherto seldom attempted. Here, we assess the relative effects of body mass and bill length, abiotic and biotic environmental factors, sex, and genome-wide ancestry on song frequency in

tinkerbirds across a hybrid zone in Southern Africa. Using path analysis, we determine the extent of direct and indirect effects of the environment and ancestry on vocal frequency. Our results confirm the strong negative effect of body mass on song frequency as well as the indirect effect of temperature via its effect on body mass. Ancestry and sex also affect vocal frequency, and we identify genes associated with frequency by performing a genome-wide association study. Yet we found little evidence for any acoustic adaptation to the vegetation structure of the environment. Overall, our study has shown that temperature-associated variation in morphology in accordance with Bergmann's rule directly affects vocal frequency in birds, but there is also a genetic component that explains further variation among species.



Heterochrony in orthodenticle expression is associated with ommatidial size variation between *Drosophila* species

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MEETING ROOM 115, August 18, 2025, 2:00 PM - 5:00 PM

Compound eye size in insects, determined by ommatidia number and size, is a key visual adaptation to diverse environments and lifestyles. Despite its ecological importance, the genetic and developmental mechanisms controlling eye size evolution remain poorly understood. We identified an X-linked region contributing to larger eyes in *Drosophila mauritiana* compared to *D. simulans*, primarily due to increased ommatidia size. We combine fine-scale mapping and gene expression analysis to investigate candidate genes within this region. We report earlier expression of the developmental gene orthodenticle (*otd*)

during ommatidial maturation in *D. mauritiana*. Functional analyses in *D. melanogaster* confirm its role in ommatidia size and organization. Furthermore, a species-specific *otd* eye enhancer recapitulates the observed heterochronic expression shift. Finally, we identify potential downstream targets of *Otd* differentially expressed between *D. mauritiana* and *D. simulans*. Our findings implicate altered *otd* expression timing in the evolution of compound eye size, providing novel insights into the genetic regulation of visual system adaptation in insects.



How to build a bigger brain?

The cellular basis of expanded mushroom bodies in *Heliconius* butterflies.

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MEETING ROOM 115, August 18, 2025, 2:00 PM - 5:00 PM

Brain expansion is a critical dimension of animal evolution, often linked to ecological innovation and behavioural complexity. Our understanding of the ways in which neurodevelopmental variation determines how neural systems are constructed is largely restricted to model species. Emerging case studies in more diverse species can now provide insights into the cell developmental pathways and patterns of genetic regulation that shape changes in neuron number, brain size and structure. One such case study are the Neotropical butterflies, *Heliconius*. Among butterflies, *Heliconius* exhibit a unique behavioural profile, as they are the only genus known to actively feed on pollen. They learn and remember the location of pollen sources, revisiting them in a spatially and temporally faithful manner reminiscent of the trap-line foraging behaviours observed in some bees. Compared to their most closely related genera in the tribe Heliconiini, *Heliconius* butterflies display enhanced visual memory, and possess expanded mushroom bodies, brain structures associated with learning and memory in insects. *Heliconius* mushroom bodies contain up to 8 times more Kenyon cells, the intrinsic mushroom body cells, than other Heliconiini, and show evidence of specialisation for visual processing. This combination of behavioural and neural specialisation makes *Heliconius* and their outgroups an excellent system for investigating the development and evolution

of nervous systems, and particularly the expansion of the mushroom body.

Using a comparative approach across two *Heliconius* and four representative Heliconiini outgroups, we generated multiple developmental time series to explore how mushroom bodies develop from newly hatched larvae to adulthood. Our time series includes 12 developmental stages. Specifically, we analysed patterns of cell proliferation within each species, by quantifying the number of progenitor cells, using immunohistochemistry, EdU labelling, and the growth of the Kenyon cell population. Our results reveal striking differences in the number of proliferating cells between *Heliconius* and non-*Heliconius* species, suggesting major changes in the cell biology of neurogenesis, and the potentially the evolution of a new intermediate progenitor cell which amplifies Kenyon cell production. Analysis of Heliconiini species with intermediate levels of Kenyon cells in adulthood suggest this was a continuous process of refining the degree of replicative division of progenitor cells. This research provides deeper insight into the mechanisms underpinning the expansion of a brain structure essential for learning and memory in insects. Currently, we are expanding this work using single-nuclei transcriptomics to uncover the molecular identity of the proliferating cells and the regulation of brain expansion.



Life in dim light: The evolution of vision in deep-sea fishes

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Background: Visual systems have evolved across diverse photic environments, from brightly-lit coral reefs to the perpetual darkness of caves and the deep sea. In vertebrates, vision is accomplished by photoreceptor cells in the retina, which detect light via visual pigments (opsins). There are two main types of photoreceptors: cones for the detection of bright light and rods for dim light vision. It is well-established that visual capabilities are adapted to ecological demands. For example, deep-sea and nocturnal species often exhibit rod-dominated retinas, sometimes to the complete exclusion of cones. In the most extreme conditions, such as in cavefishes living in complete darkness, vision can be lost altogether. Vision has been pushed to the limits of functionality in some deep-sea species, where persistent low temperatures, high-pressure conditions, and extremely limited light present unparalleled challenges for a visual system. Our work has focused on understanding how vision evolves and adapts to the extreme conditions of the deep sea.

Materials and Methods: We focused on the largest vertebrate class, fishes, employing transcriptomics, histology, and comparative genomics to investigate over 500 species, and integrating insights on developmental and evolutionary timescales. First, we studied the development of vision in deep-sea fishes using retinal transcriptomics and histology. Then, we examined the evolution of opsin genes in dim light environments, by applying a comparative genomic approach across ray-finned fishes. Finally, to understand the limits of vision in the deep sea, we examined an extreme case – the Greenland shark, a deep-sea shark which is also the longest-living vertebrate – using genomics, transcriptomics, histology, RNAscope, and in vitro opsin regeneration.

Results: Firstly, we discovered that deep-sea fishes follow a different developmental pathway to most vertebrates. Typically, during development, vertebrates initially feature a cone-dominated retina, and rods are added later. However, larval deep-sea fish possess "hybrid" photoreceptors that integrate rod and cone features into a single cell type. This finding challenges traditional paradigms of photoreceptor classification and vertebrate visual development. Next, using a large-scale genomic analysis of opsin genes, we revealed that dim light environments have consistently driven a contraction of the opsin gene repertoire across the phylogeny of fishes. Specifically, we observed highly contracted repertoires in cave-dwelling, electrogenic and deep-sea fishes. This work reveals genetic mechanisms underlying adaptive evolution in the deep sea and a striking case of convergent adaptation. Finally, we demonstrated that the Greenland shark retains an intact visual system well-adapted for life in dim light, despite having parasitized eyes, extreme longevity and a low light environment. Indeed, we found visual adaptations typical of deep-sea species, confirmed the presence of key visual cell populations, and showed that rod-specific vision genes were intact and robustly expressed. Moreover, despite being centuries old, the examined specimens showed no signs of retinal degeneration. This suggests remarkable evolutionary resilience and the maintenance of sensory function over centuries.

Conclusions: Our findings provide new insights into how vertebrate vision adapts to extreme environments via developmental, genetic and cellular mechanisms. More broadly, they illustrate how ecological pressures shape sensory evolution, offering a framework for understanding the diversification of visual systems across vertebrates.



Modality-specific memory enhancements in *Heliconius* butterflies result from specific mushroom body expansion, rather than increased investment in the sensory pathways

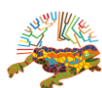
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How organisms process the world is closely linked to the species-specific demands of their ecology and life histories. Neural architecture changes have allowed species to exploit new sensory niches, facilitating evolution of huge behavioural diversity across the animal kingdom. Indeed, changes in weighting towards particular senses often results in corresponding investment shifts in the sensory processing structures. The Neotropical butterfly tribe Heliconiini offer a tractable system for studying how changes in neural circuitry have enabled adaptive shifts in behaviour. Across the short time scale of the evolution of Heliconiini, there is huge variation in the size of mushroom bodies (insect learning and memory centre). In particular, the genus *Heliconius* have greatly expanded mushroom bodies in comparison to their Heliconiini relatives. This expansion has been closely linked to shifts in behaviour and life history. This includes active pollen feeding, a dietary innovation that has afforded *Heliconius* increased lifespan and fecundity. *Heliconius* possess up to eight times more intrinsic mushroom body neurons than the other Heliconiini clades, with a notable increase in the volume of the mushroom body calyx receiving afferent visual input. This increased visual investment in the mushroom bodies is proposed to have co-occurred with evolution of *Heliconius*' systematic pollen-foraging behaviour, whereby the same pollen resources are visited with high fidelity. This behaviour is thought to rely heavily on the formation of spatial and visual-scene memories. Indeed, *Heliconius* have spatial memory and significantly enhanced stability of long-term visual memories in comparison to their outgroup Heliconiini relatives. Until now, however, it has not been known whether this cognitive enhancement in *Heliconius* was specific only to visual memories, or how their behavioural differences were accounted for in the wider neural architecture.

To address this question, I will present two studies. Firstly, we assessed modality-specific memory performance across representative *Heliconius* and outgroup Heliconiini species. Secondly, we measured differences in investment in the sensory system upstream of the mushroom bodies. In the first study, we carried out long-term olfactory memory assays in two *Heliconius* and two outgroup Heliconiini species, comparing their performance to existing visual-memory data. We found that olfactory long-term memory is present to the same degree across Heliconiini. Notably, however, comparisons between olfactory and visual memory revealed that *Heliconius* exhibit a greater retention of visual, but not olfactory long-term memories compared to outgroup Heliconiini. This indicates that selection has acted in a modality-specific manner in *Heliconius*, rather than increasing overall memory. In our second study, we used a combination of eye dissection, immunohistochemistry and neural injections to quantify investment in the sensory structures (eye morphology, visual and olfactory neuropils and the patterns of sensory projection pathways) across Heliconiini species. Overall, these analyses revealed that, although *Heliconius* have visual-specific enhancements in long-term memory, there are no concomitant increases in investment in visual architecture. In contrast, in several cases, we even found outgroup species to have higher investment in the visual components than *Heliconius*. Overall, this suggests that the massive mushroom body expansion in *Heliconius* occurred independently of the rest of the sensory processing circuitry. These results present a rare example of selection driving a highly specific and localised shift within a brain region, facilitating the evolution of a novel behavioural trait.



Neuron Number in Fish Brains: Evolutionary Insights into Computational Power

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MEETING ROOM 115, August 18, 2025, 2:00 PM - 5:00 PM

Neuronal counts serve as a critical measure for assessing brain computational capacity across vertebrate taxa, shedding light on the evolutionary trajectories of brain complexity. Yet, systematic data on neuron numbers in fish remain sparse, despite their immense diversity. This study employed the isotropic fractionator method to explore neuronal distribution in cartilaginous, lobe-finned, and ray-finned fishes, emphasizing evolutionary patterns.

Our findings highlight notable evolutionary divergence: teleost fishes, despite their compact brain architecture, exhibit remarkably high neuronal densities, particularly in the cerebellum, which houses over 50% of all brain neurons. This contrasts with lungfishes and basal ray-finned fishes, which display modest neuronal counts.

Among teleosts, benthic species demonstrate lower cerebellar neuron numbers, suggesting a correlation between habitat and cerebellar function in spatial orientation.

Intriguingly, weakly electric teleosts reveal extreme evolutionary adaptations in neuronal distribution, with cerebella holding over 95% and telencephala less than 0.5% of total neurons. Mormyrid fishes, in particular, exhibit cerebellar neuron counts rivaling those of large mammals, likely reflecting the demands of active electrolocation as a sensory modality. These findings underscore the cerebellum's role in the optimization of neural processing for complex behaviors, contributing to a broader understanding of brain evolution in vertebrates.



Phylogenetic insights into the emergence of brain anatomy and cognition.

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MEETING ROOM 115, August 18, 2025, 2:00 PM - 5:00 PM

Cortical convolutions are a hallmark of large mammalian brains, where increased brain size correlates with a steep rise in folding. These folds exhibit species-specific patterns, often mirroring the arrangement of cortical regions with distinct functions and connections. While the notion of cortical folding as a result of mechanical instability from brain growth has gained traction in the past decade, the consensus remains that folding patterns are genetically determined.

Our study explores to what extent mechanical morphogenesis shapes the development and evolution of the primate cortex. We reconstructed cerebral surfaces for more than 70 primate species, and connectomes of the underlying structural connectivity for several New World and Old World monkeys. Using phylogenetic comparative methods, we estimated ancestral states across this large variety of primate brains, and validated our findings with an independent dataset of endocasts. We developed novel methods to assess similarity of folding, cortical thickness and

connectivity patterns across species. We found that these patterns are strikingly similar between brains with similar volume no matter if they come from far branches of the phylogenetic tree. In contrast, the brains of 2 close cousins with different brain volumes show very different folding, cortical thickness and connectivity patterns.

Finally, we investigate if these mechanical processes that shape the landscape of folds and connections could also influence function and behaviour. We collected comprehensive behavioural and physiological data for all our species and found similar behavioural traits across species with similar brain volume, independent of their phylogenetic relationship.

Our findings suggest that mechanical morphogenetic processes significantly influence the organisation, anatomy, and connectivity of the primate brain, potentially impacting cognitive functions leading to the emergence of similar behavioural traits across distant evolutionary branches.



Single-nuclei transcriptomics reveals neural cell type innovation associated with mushroom body expansion in Heliconiini Butterflies.

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MEETING ROOM 115, August 18, 2025, 2:00 PM - 5:00 PM

Throughout animal evolution, the diversity of behaviour and cognitive abilities has been underpinned by evolutionary innovations and the specializations in neural systems. These innovations often arise through the acquisition of novel features built upon pre-existing neural architectures. Emerging models of brain evolution increasingly emphasise that selection acts on the local refinement of established functions—replicating and modifying existing cell types and circuits—within generally conserved networks. While current data reveal patterns consistent with neural specialisation supporting behavioural evolution, the specific mechanisms underlying cell type evolution within brain circuitry remain largely unexplored. The diversity and experimental tractability of insects make them particularly well suited for integrative studies of brain evolution within well-resolved phylogenetic and ecological contexts. Among the most variable structures in the insect brain are the mushroom bodies (MBs), major integrative centres that receive input from primary sensory regions, and are critical for associative learning and memory. MB size is highly variable across insects, ranging from ~4% to 60% of total central brain volume, largely due to changes in the number and morphology of Kenyon cells, the intrinsic MB neurons. Across insects, major increases in MB size have occurred independently multiple times, but the most recent of these expansions evolved within the Heliconiini butterflies.

The tribe Heliconiini, which includes the genus *Heliconius* which is unique among butterflies due to a suite of derived behavioural and physiological traits, including novel dietary and foraging strategies, extended lifespans, and delayed reproductive senescence. Central to these adaptations is a unique dietary shift: adult *Heliconius* actively collect and digest pollen, acquiring essential amino acids that are

unavailable to other Heliconiini. This innovation is coupled with the evolution of a spatial foraging strategy akin to trap-lining—a spatially and temporally precise strategy, also found in bees, that relies on visual memory. These behavioural specialisations are accompanied by a dramatic expansions of the MBs, including over a fourfold increase in volume relative to brain size, and up to an eightfold increase in Kenyon cell number. These expansions, primarily in visual processing regions, correlate with enhanced performance across a range of cognitive assays, including improved long-term memory and complex visual discrimination tasks.

In this study, we applied combinatorial barcoding single-nuclei transcriptomics to whole brains of *Heliconius melpomene* and *Dryas iulia* to generate comparative cell type atlases. Using three technical replicates comprising pooled whole brains and isolated Kenyon cell clusters, we generated over 300,000 high-quality single-nuclei transcriptomic profiles. Cell types were annotated semi-automatically by cross-species integration of data from five insect brain atlases. This approach enabled us to group, for each species, over 70 distinct cell types within different types of glial cells, Kenyon cells, optic lobe neurons, odorant projection neurons, and other neuron types, with just a few remaining unknown. Our analysis revealed extensive similarities in cell type identity, but with marked species-specific differences in Kenyon cell abundance and complexity. Additionally, we identified sex-biased differences in glial and optic lobe cell types, suggesting potential links to divergent mating strategies. This comprehensive atlas offers a valuable resource for investigating the evolution of neural cell types and the mechanisms underpinning behavioural innovation in *Heliconius*, and across insects.



The evolution of diversity in rhythm: Developing cross-species metrics to assess the evolutionary dynamics of rhythm and syntax in birdsong

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MEETING ROOM 115, August 18, 2025, 2:00 PM - 5:00 PM

Oscine songbirds (Order: Passeriformes) primarily use their songs for mate attraction, and their songs can evolve in response to sexual selection, with females showing a preference for males with certain song features that indicate their quality as potential mates. Previous studies have demonstrated that certain features of birdsong, such as song learning accuracy and syllable repertoire size, can signal stress incurred during early development. Females have also shown a preference towards males who are proficient at these same song traits, together suggesting that high quality learned songs are associated with greater reproductive success. Similarly, it has been hypothesized that rhythm, or the temporal patterns of songs, may function in mate choice as an indicator of fitness used by females to assess mate quality. For example, female birds in two studied species have been shown to prefer songs that are more temporally stereotyped, suggesting that rhythmicity might function in mate choice. Rhythm is an evolutionarily complex neurobehavioral trait: to produce a rhythmic song, neurons must fire at precise times in key brain regions. Practicing singing their song over time may lead to more stability and regularity of these neuronal firing patterns, and thus more rhythmic songs as a bird gets older; thus, age-dependent stability of the neural circuitry could suggest that rhythmicity may be an indicator of survivability, underscoring its potential utility as a reliable indicator of fitness. While researchers have begun investigating the temporal dynamics in birdsong in a few species, no studies have investigated how rhythm in birdsong may have changed over evolutionary history. Here, we provide new cross-species

metrics to analyze learned behaviors at scale and investigate the evolutionary dynamics of rhythm and syntax in birdsong across the Oscine clade. We developed a set of metrics to quantify rhythm and syntax in a way that is comparable across many species of songbirds, measuring 1) timing regularity using the distribution of inter-onset intervals, 2) syllable repetition by calculating the proportion of unique syllables, and 3) phrase-level repetition by measuring the compressibility of the song syntax strings. We applied these new methods to a subset of species using song recordings obtained from Xeno-canto and the Macaulay Library. Then, using Chipper, a song analysis software, we segmented the songs into syllables and obtained data necessary for the temporal metrics, and we also obtained the syntax of the songs manually for our syntax repetition metrics. We calculated the median values of each trait for each species to perform phylogenetic comparative analyses. Interestingly, we found that the rhythm traits of our three metrics have similar predicted ancestral states and evolve in similar patterns to one another, largely following a Brownian motion model of evolution (Blomberg's $\kappa = 0.826 - 1.051$). While closely related species tend to have more similar rhythm patterns, rhythm is not perfectly conserved, suggesting that there might be other factors, such as ecology or life history, that may be driving the evolution of rhythmicity. We have conducted preliminary phylogenetic analyses to assess the effects of other selective pressures on the patterns of rhythm evolution across the Oscine songbird phylogeny, providing the first in-depth analysis of rhythmicity on a phylogenetic scale, including the pace of change over time and in relation to life history and other traits.



The genetic basis of neural circuit evolution for *Drosophila* mate preferences

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MEETING ROOM 115, August 18, 2025, 2:00 PM - 5:00 PM

Behaviors that arbitrate interspecies courtship are at the crux of evolutionary barriers between species. Species- and sex-specific pheromone profiles in *Drosophila* have rapidly diversified that signify species' identity to encourage courtship among conspecifics and discourage interspecies courtship. The dominant pheromone produced by *Drosophila melanogaster* females is an aphrodisiac to their males, but the same chemical cue inhibits courtship in male *D. simulans*, whose females do not produce that pheromone. We identify a gene that has evolved to reshape the neural circuit that processes this pheromone and change

the male's response from attraction to repulsion. We assess how this gene changes the anatomical and physiological properties of specific neurons in the circuit between the species to produce divergent behaviors. Additionally, we examine intra-species variation in genetic and environmental factors that alter female production of and male attraction to the pheromone cue. Together, this provides a unique mechanistic understanding of the genetics underlying neural circuit evolution and how the corresponding behavior affects evolutionary processes in the wild across different timescales.



Visual System Adaptations Underlying the Evolution of Flower-Visiting Behavior in *Drosophila*

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MEETING ROOM 115, August 18, 2025, 10:30 AM - 12:30 PM

The evolution of animal behavior can generate novel interspecies interactions, potentially reshaping ecosystems. One notable example is the emergence of flower-visiting behavior in insects, which has established new animal-plant interactions. Insects appeared long before flowering plants evolved and later developed flower-visiting behaviors following the adaptive radiation of angiosperms. Today, flower-visiting insects are responsible for pollinating approximately 90 percent of angiosperm species, playing a vital role in maintaining terrestrial ecosystems. However, the mechanisms by which this behavior evolved in insects, and specifically what novel brain functions and neural systems enabled this transition, remains largely unknown. To address this question, we focused on *Drosophila elegans*, a species that has acquired flower-visiting behavior for breeding and feeding, diverging from its fruit-breeding ancestors. Because *D. elegans* is amenable to genome editing and closely related to the non-flower-visiting model organism *D. melanogaster*, it serves as a useful model for investigating the neural mechanisms underlying the evolution of flower-visiting behavior.

D. elegans preferentially visits blue *Ipomoea* flowers in the wild. To identify the sensory modalities involved, we analyzed behavior of newly emerged flies under controlled conditions. *D. elegans* was found to preferentially visit artificial *Ipomoea*-like fabric flowers, photographs of *Ipomoea* flowers, and even blue circular paper disks presented

with green, beige, and yellow disks with similar brightness all lacking any floral scent. These findings suggest their strong reliance on vision, particularly color cues, for flower recognition. Notably, *D. melanogaster* did not visit any colored disks under the same experimental conditions, implying that brain functions related to visual and color information processing are crucial for the evolution of flower-visiting behavior.

To understand how visual processing systems may have changed during this behavioral evolution, we compared the visual systems of the two species. Transmission electron microscopy revealed that the rhabdomeres of R8 photoreceptor cells, which are sensitive to blue or green light depending on the ommatidial subtype, are larger in *D. elegans*. Electroretinogram recordings further showed that *D. elegans* exhibits relatively higher sensitivity to blue light. These findings suggest that *D. elegans* has evolved a visual system adapted to detect blue *Ipomoea* flowers. Genome editing experiments demonstrated that R7 and/or blue-type R8 photoreceptor cells are essential for this flower-visiting behavior. Together, our results indicate that evolutionary changes in the neural system, beginning with these photoreceptors, played a key role in the emergence of flower-visiting behavior. Since many flower-visiting insects rely on visual cues, our findings provide novel insights into the brain functions and neural adaptations contributing to the evolution of flower-visiting behavior.



Why did the human brain size evolve? A way forward

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MEETING ROOM 115, August 18, 2025, 2:00 PM - 5:00 PM

Why the human brain size evolved has been a major evolutionary puzzle since Darwin but addressing it has been challenging. A key reason is the lack of research tools to infer the causes of a unique event for which experiments are not possible. We suggest that analogous problems have been successfully addressed in other disciplines using what has been recently termed simulation-based inference. Following that approach, we outline a strategy to address why the human brain size evolved: hypotheses are expressed in mechanistic models that yield quantitative predictions for evolutionary and developmental trajectories of brain and body sizes, the predicted trajectories are compared to data, and

models are chosen by their ability to explain the data. We discuss a recently published model that makes quantitative predictions for evolutionary and developmental trajectories of brain and body sizes for six hominin species and compare the model predictions to data, finding that the model recovers many aspects of hominin evolution and development. Counter-intuitively, the human brain size evolves in this model as a spandrel or by-product of selection for something else, namely, fertility-determinant traits. Our analysis indicates that simulation-based inference offers a way forward to infer why the human brain size evolved.





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S18 - Evolution of biotic interactions across scales

A la carte seed harvesting: *Messor barbarus* selects durum wheat genotypes

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POSTER SESSION 1 - P01.230, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Biotic interactions between plants and insects can significantly shape their reciprocal evolution. Seed predation by ants can be viewed as either a mutualistic or antagonistic interaction, depending on the balance between its costs and benefits for the plant species. In Mediterranean agroecosystems, harvester ants specialize in collecting seeds, including those of cultivated cereals. Yet their potential role in shaping crop traits remains largely unexplored. Durum wheat (*Triticum turgidum* ssp. *durum*), a key Mediterranean crop domesticated from wild emmer (*Triticum turgidum* ssp. *dicoccoides*), has undergone major morphological changes during domestication, particularly in reproductive organs such as spike and seeds. However, whether interactions with seed predators like harvester ants contributed to the selection specific plant phenotypes remains unknown. In this study, we investigate the genetic architecture underlying durum wheat susceptibility to ant predation, and then test in a separate experiment whether *M. barbarus* foraging behavior reflects plant genetic variation. Building on observations from a field experiment, we investigated the phenotypic and genetic determinants of seed predation by the harvester ant *Messor barbarus* across a panel of 180 durum wheat inbred-lines exhibiting high phenotypic and genetic diversity. First, we visually assessed spike predation by *M. barbarus* on 95 genotypes within ant foraging areas and conducted a genome-wide association study (GWAS) using SNP markers to uncover the genetic basis of harvester ant predation on durum wheat. Based on the identified genomic regions, we examine the functions of candidate genes potentially involved in ant predation. We also designed a cafeteria experiment using 208 spikes from 26 genotypes placed at the entrance of eight nests to validate our genetic findings and

investigate how spike and seed traits influence ant preferences. We estimated the heritability of wheat on ant preference using the spike mass loss after 24 and 42 hours and tested whether ant preference is partly genetically determined in wheat. Then, we analysed the selective pressure exerted by ants on spike and seed traits, such as morphological characteristics and protein content, to better understand the selective pressures at play.

We detected a significant Quantitative Trait Loci (QTL) on chromosome 2A, whose allelic variation explained 21% of the observed phenotypic variation in predation rates by *M. barbarus*. This region encompasses a 3.6 Mb segment that includes a chromosomal inversion between wild emmer wheat and durum wheat, and 46 candidate genes, from which one MYB transcription factors that could be involved in cuticle and chemical trait regulation. This QTL was validated by the cafeteria experiment: ants preferentially removed spikes from genotypes carrying the preferred allele detected from the GWAS analysis. Furthermore, ants preferentially started to harvest seeds from shorter spikes, with some ants sometimes bringing the whole spikes into the ant nest. While spike morphology did affect ant choice, we did not find any significant correlation between seed morphology or protein content and predation levels.

Our study provides the first evidence that harvester ants exhibit genotype-specific preferences in durum wheat, and that these preferences are linked to a major QTL on chromosome 2A. These findings suggest that ant foraging may be an underappreciated factor in cereal crop evolution, providing new perspectives to better understand interactions between seed predators and crops.

Altruism or Selfishness? Evolution of floral behavior in response to neighboring kinship.

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POSTER SESSION 1 - **P01.199**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Kin recognition in plants may induce behavioral plasticity, including changes in floral traits. Recent studies have shown that plants growing with genetically related neighbors tend to produce larger floral displays than those with unrelated individuals. This pattern has been interpreted as altruism, where plants incur the cost of larger flowers to attract more pollinators to the patch, thereby increasing the pollination success of neighboring kin. However, whether such behavior consistently represents altruism remains unclear.

Materials and methods: We developed an evolutionary model in which plants adopt one of two floral display tactics depending on the genetic relatedness of neighboring individuals. The model incorporates ecological parameters such as pollinator foraging preferences for large flowers and plant dispersal ability. We used individual-based simulations to explore the evolutionary outcomes of these plastic floral strategies.

Results: Our results predict the evolution of two distinct strategies facilitated by kin recognition: altruistic behavior involving increased floral display toward kin, and a free-rider strategy involving reduced floral display toward non-kin. Increasing floral display toward kin evolved when pollinators showed weak preference for individual plants with large floral displays or when kin

recognition was highly accurate. Under these conditions, larger floral displays enhanced group-level pollinator attraction, thereby increasing the pollination success of neighboring individuals—consistent with altruistic behavior. In contrast, reducing floral display toward non-kin evolved when pollinator preferences for individual large-flowered plants were weak. This strategy can be interpreted as a free-rider strategy, allowing individuals to save the cost of producing and maintaining their own floral displays while still benefiting from pollinators attracted by neighbors' flowers.

Conclusions: Our findings indicate that the observed tendency for plants to produce larger floral displays when growing with kin, compared to non-kin, may arise from three distinct mechanisms: (1) altruistic behavior toward kin, (2) free-rider strategies toward non-kin, or (3) a combination of both. Thus, differences in floral traits between kin and non-kin alone do not provide conclusive evidence of altruism. Interpretation requires consideration of ecological and evolutionary contexts.

This research has been published in the *Journal of Evolutionary Biology*:

Tomizuka, H., Yamawo, A., & Tachiki, Y. (2025). Altruism or selfishness: Floral behavior based on genetic relatedness with neighboring plants. *Journal of Evolutionary Biology*, voaf015. <https://doi.org/10.1093/jeb/voaf015>

Butterfly counter-adaptations against multiple plant defences: arms race, plasticity-first and convergent evolution

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POSTER SESSION 1 - **P01.233**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The toxic and colourful butterflies of the Heliconiini tribe (~80 species) sequester cyanogenic glucosides from their obligatory Passiflora hostplants. To escape Heliconiini herbivory, some Passiflora species have disabled sequestration by diversifying their cyanogen structure, whereas others have invested in physical defences, such as hooked trichomes

Using larval performance assays, I have demonstrated that Heliconiini butterflies increase de novo biosynthesis of cyanogens, when compounds for sequestration are not present in their Passiflora hosts. This biochemical plasticity allows them to keep their toxicity despite the cyanogen profile of their hostplant. Using target-metabolomics data of over 700 Heliconiini butterflies from different populations, we performed ancestral state reconstruction analysis to show that biochemical plasticity was ancestral in this tribe, but has been lost in multiple lineages. We also used CRISPR to characterize the genes involved in cyanogen biosynthesis in Heliconiini (CYP405 AND CYP332). These genes are incomplete in sapho clade which is specialized in sequestration and might have lost biochemical plasticity. This therefore

represents an example of 'plasticity-first' evolution, whereby plasticity has enabled colonisation of a diversity of hostplants and then been lost as species specialise ecologically. Interestingly, Heliconiini butterflies and Zygaena moths, which also feed on cyanogenic plants, have independently coopted the same cytochrome P450s into cyanogen biosynthesis.

Moreover, we also have found phenotypic plasticity in the Heliconiini counter-adaptations to plant physical defences. We have shown that the hooked trichomes found in some Passiflora species are effective against most Heliconiini caterpillar due to its sharpness and unique elemental composition. Among other heliconiines, Heliconius charithonia is the only one that has overcome this physical defences by evolving a thicker cuticle and a more sclerotized gut. While their external counter-adaptation is constitutive, their internal counter-adaptation is inducible and have high fitness costs.

Our results emphasize the importance of phenotypic plasticity for coevolution and exemplify how plasticity can contribute to diversification. It also brings new knowledge on a text-book example of coevolution

Community tipping driven by predator-prey role reversal in a fishing-induced trophic cascade

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POSTER SESSION 1 - P01.215, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Top predators worldwide have experienced dramatic declines due to anthropogenic pressures such as overexploitation and habitat loss. Such catastrophic population collapses often trigger trophic cascades where mid-sized predatory species increase in abundance – a phenomenon known as mesopredator release. This may increase interspecific competition for resources or even reverse traditional predator–prey relationships, where mesopredators intensively prey on the early life stages of top predators, suppressing recruitment and potentially hindering population recovery. These drastic changes in community composition are thought to represent transitions between distinct, stable configurations of the system, known as alternative stable states. Such transitions or “regime shifts” typically occur when the system crosses critical thresholds, referred to as “tipping points”. In addition, human-induced stressors may trigger evolutionary responses in top predators, as indicated by the rapid changes in phenotypic traits of wild fish populations such as the size at maturation and reproductive investment, due to overharvesting. However, while these ecological and evolutionary changes often occur simultaneously, their joint dynamics and interactive effects on communities are poorly understood. To address this gap, we

build a multispecies ecological model with population structure, size- and food-dependent consumer growth and size-selective predation mortality. The food chain model consists of a size-structured predator that forages on a size-structured consumer, which, in turn, feeds on an unstructured basal resource. We investigate the effects of predator-prey role reversal on tipping in this system by varying the dietary preference of the adult mesopredator between the resource and the juvenile top predator. Additionally, we explore whether the rapid evolution and/or phenotypic plasticity of relevant life-history traits of the top predator, and their interaction, affect the risk, timing, and abruptness of regime shifts under various scenarios of fishing mortality. We formulate distinct model variants based on two trophic networks where the top predators have been heavily exploited: the Atlantic cod and the northern pike in the Baltic Sea, and parameterise the models using existing metabolic and life history data. We demonstrate that the eco-evolutionary dynamics of a fundamental biotic interaction under global change can drive regime shifts with dramatic consequences for trophic dynamics, community structure, and ecosystem functioning.

Divergent Defence: Multiple Evolutionary Routes of Algal Resistance and Their Costs

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POSTER SESSION 1 - P01.223, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The evolution of host resistance initiates coevolution between hosts and parasites, thereby profoundly shaping both host communities and ecosystem structure. It is an important aspect that hosts can develop multiple resistance mechanisms to combat specific parasites. If different resistance mechanisms target different stages of infection, this could affect the further course of coevolution. Moreover, different defences may come with different costs, affecting host life-history traits and population dynamics. This study uses an alga-virus model system to investigate divergent host evolution, which could influence the subsequent course of host-parasite coevolution. In our previous work with the green alga *Chlorella variabilis* and virus PBCV-1, we generated a panel of 22 resistant algal clones that independently evolved resistance to the virus. Preliminary data suggest that these resistant clones can

have at least three distinct resistance mechanisms. Here, we will present the first data assessing whether the resistant *Chlorella* clones have different resistance mechanisms. Specifically, we will test whether algal clones show resistance by blocking different stages in the virus cycle: virus adsorption to the algal cell, depolarization and subsequent genome entry, or the release of progeny virions. Furthermore, we will investigate whether the initial cost of achieving this resistance varies among the resistant algal clones. This work will be the first information on diverse resistance pathways of *Chlorella* algae and lays the groundwork for understanding how variation in host resistance mechanisms can influence the evolution of hosts and parasites.

Ecological aspects of coinfections: Do laboratory experiments of within-host parasite interactions reflect the reality of coinfections in nature?

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POSTER SESSION 1 - P01.227, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: In nature, organisms are commonly found to be infected by more than one parasite species, and many studies have demonstrated that parasites interact with each other in various ways, affecting each other's fitness within and between hosts. Yet our knowledge is mostly about interactions within individual hosts and their parasites, and our understanding of the role coinfections have at other scales (e.g., host populations and parasite communities) is far from being conclusive. More precisely, do laboratory experiments of within-host parasite interactions reflect the reality of coinfections in nature?

Materials and methods: We used the *Daphnia*-microparasites system to ask if within-host interactions among parasites cascade to patterns at the host population scale. To this end, we first studied in detail the interactions between the fungus *Metschnikowia bicuspidata* and the microsporidium *Hamiltosporidium tvaerminnensis* within individual *Daphnia magna*. Then, we studied the same combination of parasites in microcosm host

populations and asked if within-host interactions manifest themselves in the prevalence and density patterns of the parasites and the host. Finally, we studied the temporal dynamics of coinfections in natural host populations and asked whether the prevalence of coinfections, in general and of different members of the parasite community, hints on the, usually negative, interactions among coinfecting *D. magna* parasites.

Results: While within-host interactions indicated strong competition between the two species, with negative consequences for the host, their reflection in the microcosms patterns was weak. Furthermore, in natural populations coinfections were completely random and reflected the diversity of the community of parasites.

Conclusions: While the importance of coinfections is large at the individual scale, it decreases at more complex scales, where they are potentially diminished by other factors as well as by stochasticity.

Ecological conditions can promote female-biased sex-ratios: a case study in haplodiploid gregarious pine sawfly

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POSTER SESSION 1 - **P01.207**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Ecological conditions can result in variation in selection pressure for mothers to produce the more helpful sex (i.e. local resource enhancement hypothesis). We tested this hypothesis with the gregarious pine sawflies (Hymenoptera), which rely on cooperative chemical defence in larval stage. Contributing to the defence is costly for an individual and individual's survival is expected to be higher in groups where a larger proportion of larvae produces the defensive secretion. Our data from wild populations show that sex-ratios in larval groups can vary from all-male to all-females. However, on average sex-ratios are female-biased both on group and population level. To study the benefits of having females in the larval group, we quantified the fitness and cooperative behaviour of individuals in virgin produced all-male groups and sexually produced mixed sex groups under low resin (control) and high resin content (harsh

environment) host plant diets (*Pinus sylvestris*). We also tested whether reproductive output differs between virgin and mated females. We found that cooperativeness was sex-biased: haploid male larvae contributed less to the group defence than females. Interestingly, males benefitted from the presence of their sisters especially in harsher conditions as males grew faster on a high resin diet in mixed-sex groups in comparison to all-male groups. In addition, we found that reproductive output was higher in mated than in virgin females suggesting that mating increased fertility of females. Altogether, these results suggest that local resource enhancement together with the benefits of sexual reproduction may promote female-biased sex-ratios. The role of the social structure of the group in the invasion success of gregarious herbivorous insects will be discussed.

Enhancing plant disease resistance through intra and interspecific diversification

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POSTER SESSION 1 - P01.211, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Agricultural intensification has led to a significant reduction in cultivated biodiversity, with global food production increasingly dependent on a limited number of species. This trend has contributed to climate change, biodiversity loss, and human health concerns. One major consequence of crop genetic homogenization is the increased vulnerability of agricultural systems to pests, diseases, and weeds at both field and landscape levels. In contrast, diversification has emerged as a promising strategy for sustainable, pesticide-free agriculture by enhancing resilience and stability. At the national scale, diversification broadens crop variety, while at the farm level, techniques such as extended crop rotations, cover cropping, intercropping, and cultivar mixtures improve pest and disease control.

The French collaborative project MoBiDiv explores the role of diversification in influencing the establishment and evolution of plant diseases, pests, and weeds. Specifically, we aim to assess how diversification enhances the durability of disease resistance and alters disease susceptibility within mixed cropping systems. Additionally, we seek to determine whether intra-plot diversification drives genetic co-selection between interacting plants.

To evaluate the impact of intercropping on fungal disease incidence, we conducted field trials using three intercropping systems: (i) wheat-alfalfa, (ii) wheat-perennial prairie, and (iii) wheat-pea. Disease assessments focused on key fungal pathogens, including septoria tritici blotch and brown rust in wheat, and ascochyta blight and anthracnosis in pea.

Our findings demonstrated that intercropping effectively reduced disease severity, particularly in anthracnosis affecting pea. However, no significant effect was observed on wheat fungal diseases in the wheat-pea system, while septoria incidence was lower in wheat intercropped with prairie. This highlights the potential of certain intercropping combinations in mitigating specific disease pressures.

To further investigate the genetic mechanisms underlying these effects, we analyzed potential co-selection between plants that might influence susceptibility to fungal diseases in wheat intraspecific mixtures. We conducted the first co-Genome Wide Association Study (co-GWAS) between two interacting plants and tested whether loci involved in inter-individual interactions exhibited signatures of selection. We calculated Tajima's D values to detect selection traces, expecting co-selected loci to show more negative values than control regions. Linkage disequilibrium (LD) was also assessed between interacting loci, hypothesizing that co-selected regions would exhibit high r^2 values. Finally, we compared intra- and inter-subgenome interactions, postulating that co-selection would primarily occur within sub-genomes in ancestral wheat lines predating polyploidization. Our analyses did not detect clear evidence of co-selection. This suggests that intra-plot diversification influences disease suppression primarily through eco-physiological mechanisms rather than co-evolutionary genetic adaptation. Understanding the impact of these underlying mechanisms on disease reduction is important to optimize diversification strategies and resistance management.

Evolution of coexistence ability in experimental *Daphnia* mesocosms

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POSTER SESSION 1 - P01.217, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Understanding species coexistence is central to community ecology, given its role in biodiversity maintenance. Theory indicates that coexistence is determined by niche and fitness differences, i.e. differential resource use or other stabilizing mechanisms and relative competitive abilities of species. Both of these can evolve as a result of selection in underlying quantitative traits, and thus potentially alter coexistence dynamics. However, it remains unclear how adaptations to competitors develop over time and how abiotic environmental properties influence dynamics, shaping evolutionary trajectories of competition and coexistence. We conducted a multi-year, semi-natural outdoor mesocosm experiment with planktonic communities to study different selection pressures as drivers for evolution of coexistence. The mesocosms used realistic annual hydroperiods, which better allows us to determine whether rapid evolution observed within a growing season persists when genetic drift and sexual reproduction are considered, and also allows for eco-evolutionary feedback dynamics between phytoplankton and zooplankton. To generate distinct evolutionary histories, focal zooplankton species *Daphnia magna* and *Daphnia pulex*

were included either in monoculture or in competition into mesocosms as initially diverse populations (22 unique clones). Three years after the initial implementation, we tested how evolution acted on niche and fitness differences of focal *Daphnia* species.

Niche and fitness differences were measured in laboratory experiments, using recent methodology that recommends measuring distinct intrinsic, invasion, and no-niche (when niches are equivalent between the two species) growth rates. We hypothesized that *Daphnia* from competition treatments would evolve either increased niche differentiation and/or reduced fitness differences in competition treatments, facilitating coexistence by reducing direct competition or stabilizing effects. Our results showed evidence for reduction of fitness differences among *Daphnia* populations with histories of coexistence, but no consistent directional shifts in niche differences were observed. These findings indicate that *Daphnia* evolve mechanisms that impact coexistence in different, repeatable directions when the competitors are present or absent, and a next critical step will be to determine which life history traits drive the reduced fitness inequalities observed in populations exposed to competition.

Evolution of plasticity and character displacement in a fluctuating environment

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*POSTER SESSION 1 - **P01.193**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM*

Species that compete for the same resource may undergo ecological character displacement (CD), where the phenotype of each species evolves to deviate from its optimum in the absence of a competitor, thus reducing the intensity of competition. As natural habitats are rarely static, interspecific competition is likely to occur in environments that fluctuate over time. Such environmental fluctuations can in turn cause the evolution of phenotypic plasticity for traits mediating the competition. However, the interplay of environmental fluctuations, evolution of plasticity, and character displacement, has been little investigated. We use a quantitative genetic model to study theoretically how a randomly fluctuating environment and evolution of plasticity influence the outcome of ecological CD between two competing species. We show that environmental fluctuations make the

conditions for CD more stringent, requiring stronger competitive selection relative to stabilizing selection. This occurs because environmental fluctuations reduces the average population size, and thereby competition intensity. Evolving plasticity can restore CD by buffering the impact of environmental fluctuations through phenotypic tracking, to a degree that depends on environmental predictability. Somewhat paradoxically, competition that favors phenotypic divergence among species can cause convergence in plasticity, when this reduces the load caused by fluctuations in phenotypic divergence. Our results shed light on how competition and plasticity influences evolution of the fundamental niche in a fluctuating environment.

Examining the relationship between genomic variation and avian cholera survival in an Arctic sea duck

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POSTER SESSION 1 - P01.209, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Conservation efforts are urgently needed for Arctic species, as climate change allows diseases to spread farther north. However, critical information on the genetics of disease resistance is lacking. The Common Eider (*Somateria mollissima*) is an Arctic sea duck and is an important source of fresh protein and clothing insulation for northern and coastal communities. In 2006, avian cholera killed > 30 % of female nesting eiders at the largest breeding colony in the eastern Arctic (Mitivik Island, Nunavut, Canada).

Materials and methods: We are using whole genome sequencing and cutting-edge bioinformatic approaches to test for a genetic basis for resistance to cholera in eiders, utilizing an extensively archived time-series of DNA from the Mitivik Island colony to compare variation before and after the cholera epizootic. Using these whole genome sequences, we identified single nucleotide polymorphisms (SNPs) at genes previously found to be involved in immunity in other species. We tested whether this variation was associated with inter-individual variation in the eiders' mortality from cholera.

Results: We found that diversity of genes previously found to be involved in immunity (e.g., toll-like receptors or TLRs) was related to eider survival during the cholera epizootic. For example, heterozygosity at two genes, TLR5 and TLR3, was related to whether eiders

that were exposed to cholera survived. Additionally, we identified specific immune gene SNPs that were associated with eider survival during the cholera epizootic and found evidence of selection on SNPs in these genes. Specifically, several SNPs in TLR2B and TLR21 were also associated with disease survival. Considering only non-synonymous SNPs changed the results: heterozygosity at TLRs 2B, 3, 7, and 21 was associated with eider cholera survival. Additionally, several SNPs in TLR2B and TLR21 were associated with eider cholera survival.

Conclusions: These results suggest that immune gene variation affects resistance of wild Common Eiders to avian cholera. Additionally, we found that considering only nonsynonymous SNPs identified different genes putatively involved in disease immunity compared to considering all SNPs. One potential explanation is that synonymous variation may have masked the role of nonsynonymous variation. This highlights the importance of considering functional genetic variability separately from total genetic variation. Our work adds to a small but growing body of literature regarding the population genetics of disease resistance in wild populations. Our findings inform the genomic impacts of emerging infectious diseases and the potential for species adaptation, and therefore the vulnerability of Common Eiders and Arctic species in general to emerging pathogens.

Experimental Removal of Niche Construction Alters the Pace and Mechanisms of Resistance Evolution

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POSTER SESSION 1 - P01.196, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Biotic interactions can strongly depend on ecological conditions. In turn, ecological conditions can be influenced by the biotic interaction partners themselves. Specifically, niche construction is a central eco-evolutionary process in which organisms modify their environment. Such modifications have the potential to profoundly affect hosts and their biotic interaction partners. However, experimental knowledge how niche construction affects the evolution of biotic interactions is currently lacking.

Red flour beetles modify their environment by releasing quinone-rich stink gland secretions, a form of external immunity. We tested in an evolution experiment how experimental removal of niche construction—using RNAi of a key gene needed to produce stink gland secretions—affects the beetles' adaptation to the bacterial entomopathogen *Bacillus thuringiensis*. In each generation, beetles inhabited conditioned flour either with

regular or drastically reduced stink gland secretion content. Compared to the non-exposed control, all bacteria-exposed regimes rapidly evolved bacterial resistance within only six generations. However, beetles evolving with intact niche construction acquired resistance as soon as after three generations, indicating that adaptation was initially facilitated by niche construction. RNAseq of evolved beetles showed that gene expression differed strongly between the selection regimes, revealing that the mechanisms underlying resistance were dependent on niche construction.

After another nine generations of experimental evolution, we further analysed whether larvae-associated microbiota or the beetles' secretions had changed and found that lines evolving with the possibility to construct their niches showed increased microbial diversification. In sum, our findings provide much-needed empirical evidence on the role of niche construction for resistance evolution and its potential genetic basis.

Exploring Density-Dependent Shifts in Symbiotic Interactions Using *Paramecium bursaria* and *Chlorella* Species

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POSTER SESSION 1 - P01.214, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The endosymbiosis between ciliate hosts and endosymbiotic algae have evolved independently several times. These relationships are often nutrient mutualisms, where ciliates provide nitrogen to the algae and the algae provide carbon to the ciliates, resulting in a net positive effect for both partners. However, the net effect of the interaction can shift depending on environmental conditions, although little is known about when and why these shifts occur. In our study, we investigate when the balance of costs and benefits of one or both symbiosis partner shifts so that the net effect on each partner changes from positive to negative or vice versa. We use *Paramecium bursaria* as a host and test its interactions with several closely related green algae of the genus *Chlorella*, most of which are capable of forming facultative endosymbiotic relationships with the ciliate. By measuring how costs and benefits vary under different environmental conditions,

such as changes in light, nutrient availability, and host and symbiont densities, we assess the context-dependence of the symbiosis. Our results show that as *P. bursaria* density increases, the net effect of the endosymbiotic relationship often shifts from positive to negative, and this shift depends on the specific *Chlorella* species involved. By teasing apart how costs and benefits respond to changing conditions, we begin to understand why these relationships change and what this means for stability of the symbiosis. Overall, our results suggest that the relationship between *P. bursaria* and *Chlorella* species can range from mutualism to exploitation, depending on environmental factors and the identity of the algal partner. Understanding how these conditions shape the dynamics of endosymbiosis will provide important insights into its ecological role and how such interactions evolve and persist over time.

Exploring the evolutionary dynamics of mating system shifts and speciation in *Capsella*

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POSTER SESSION 1 - **P01.221**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Most flowering plants are obligatory outcrossers, which means that they rely on pollen vectors for reproduction. This type of reproduction entails a self-incompatibility system that prevents inbreeding. Repeatedly in plant evolution this system broke down allowing reproduction through self-fertilization. This transition from outbreeding to selfing often leads to significant morphological and functional changes, including reduced flower size, altered sex allocation, and a decreased distance between anthers and stigma. These convergent phenotypical traits are known as the selfing syndrome. Selfing species also become quickly isolated from their outbreeding ancestors, but it remains unclear what genetic and ecological mechanisms reinforce isolation between newly evolved selfers and their outcrossing progenitors.

To address this, we focused on the *Capsella* genus (Brassicaceae) which emerged as an ideal model for studying the transition from outbreeding to selfing. In this study, we investigated how the incipient selfing lineage *Capsella rubella* (Cr) (50,000 years ago) became reproductively isolated from its outcrosser ancestor *Capsella grandiflora* (Cg).

We investigated how different barriers to gene flow affect reproductive isolation. After a survey to find populations that live in sympatry, we discovered that hybrid formation is possible in nature but limited. Interestingly, the number of hybrids mothered by Cr plants is higher than the ones in Cg plants. This directional gene flow was further confirmed by admixture analysis, in which we observe higher introgression rates from Cg to Cr but lower in the opposite direction.

Hybrid disgenesis caused by the developmental failure of interspecific seed nutritive tissues is prominent between the two species. Such defects in seed formation are attributed to

differences in the intensity of parental conflicts between outcrossing and selfing lineages, itself driven by variation in intrasexual competition. This result suggests that the low hybridization rate could trigger reproductive interference, thereby promoting isolation before fecundation. Pre-zygotic barriers are more effective in preventing resource waste derived from reproductive interference, as they prevent fertilization and, hence, gene flow. Flowering phenology is mostly overlapping, but characteristics related to pollinators' attraction limit cross-pollination. In fact, we observe the typical flower morphology associated with the selfing syndrome in Cr: reduction in flower size, pollen number, volatile production, limited pollinator visits and gene flow. In particular, the striking difference in pollen number, higher in Cg and lower in Cr, greatly limits the possibility of Cr pollen to reach Cg pistils. However, the higher Cg pollen production, due to high male competitiveness, increases the possibility of pollination of Cr. Such promotion of interspecific directional gene flow, likely explain higher hybrid mothering by Cr and introgression rate from Cg to Cr. In Cr, early self-pollination nevertheless significantly reduces interference from Cg pollen.

Lastly, post-pollination prezygotic barriers, like pollen compatibility and efficiency, seem to be relatively weaker. Nonetheless, we observe partial germination of interspecific pollen on both pistils. Cg pollen outcompetes Cr pollen in fertilizing ovules, suggesting a difference in pollen tube growth, probably due to enhanced male competitiveness in outcrossers.

Our work clarifies how the interplay of different selective pressures shapes barriers to gene flow between lineages with contrasting mating systems.

From antagonists to mutualists: the emergence of brood-site pollination in Ochyromerini weevils revealed by a new weevil specific UCE probe set.

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POSTER SESSION 1 - P01.212, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

As phytophagous insects, weevils (Coleoptera, Curculionoidea) are mostly known for their antagonistic relationships with plants. Yet, examples of mutualistic interactions between weevils and plants are being revealed by a growing number of publications. Recent research showed that transitions from antagonism to mutualism emerged at least a dozen times in the evolutionary history of weevils in the form of brood-site pollination. Each of these evolutionary events makes up an ideal repeated pattern to study the emergence of mutualistic interactions. In this context, a robust phylogenetic background for weevils is necessary. Thankfully, the insect tree of life is being continuously pushed forward thanks to the widespread use of ultraconserved elements for phylogenomic analyses. However, despite previous efforts, the evolutionary history of weevils, has yet to be confidently resolved due to insufficient phylogenetic signal in currently available probe sets. To solve this issue, we designed a new set of probes specific to Curculionoidea with a novel method based

on genomes alignment. Genetic markers obtained with this method offer better phylogenetic support at wide and shallow taxonomic levels than single reference based methods commonly used today. Using this new set of UCE, we took advantage of museum specimens to reconstruct the first phylogenetic tree of one major and broadly understudied group of brood-site pollinating weevils: the Ochyromerini tribe. Combined with what we know of their ecological interactions, these results shed light on the systematics of the tribe and the pattern of emergence of mutualism at the species scale. Ongoing research will focus on many other aspects of their evolutionary history, such as their biogeography, diversification rates and host plant associations dynamics. While we are planning to do the same with other pollinating weevil lineages, we hope that sharing this new set of Curculionoidea baits along with the methods utilized to generate and analyse them will allow to unveil the deeper radiation of the most diverse group of insects known so far.

From spore to infection: How the intensity and frequency of heatwaves shape bacterial infections of a crustacean

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POSTER SESSION 1 - P01.200, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Host-parasite interactions are key components of ecosystems, yet their dynamics are increasingly shaped by climate extremes such as heatwaves and drought. While elevated temperatures are known to affect infection traits in both hosts and parasites, little is known about how climate stress impacts parasites during their off-host stages. We investigated how heat exposure affects the free-living spores of the bacterial parasite *Pasteuria ramosa*, which infects the freshwater crustacean *Daphnia magna*. Spores were subjected to a range of elevated temperatures (20-60°C) and durations (1-21

days), simulating realistic heatwave conditions, then used in infection assays. Surprisingly, heat-treated spores retained their ability to attach to hosts, but failed to complete infection—indicating that thermal damage occurs after attachment, disrupting later stages of the parasite's development. These findings reveal a hidden vulnerability in parasite life cycles and underscore the need to incorporate off-host stages into predictions of disease dynamics under climate change.

Host-parasite coevolution in complex natural systems: linking immune gene variation to infection patterns across amphibian species

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POSTER SESSION 1 - P01.222, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The importance of coinfection in host-parasite coevolution is poorly understood, especially for non-model organisms and complex natural systems (i.e. multihost-multiparasite). This knowledge is urgently needed for endangered taxa such as amphibians, since infectious diseases play a major role in their dramatic decline. Immune gene variation, particularly in the major histocompatibility complex (MHC), offers a powerful tool to explore the evolutionary dynamics between hosts and their parasites, as it reflects selective pressures imposed by infections. In this study, we adopt a multi-scale framework combining MHC class I and II genotyping from over 1000 individuals across 20 European amphibian species with

infection data for seven parasite taxa. Using generalized linear mixed models and phylogenetically informed analyses, we investigate associations between immune gene diversity at both individual (infection status and parasite load) and population scales (prevalence and load). By embracing the ecological complexity of multihost-multiparasite networks, our integrative approach offers new insights into host-parasite coevolution in natural ecosystems. To our knowledge, this is the first study to apply such an approach to amphibian populations, providing a crucial step toward understanding and mitigating their ongoing biodiversity loss.

Is a generalist parasitic plant locally adapted to its plant hosts?

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*POSTER SESSION 1 - **P01.203**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM*

Parasitism has a major role in the evolutionary process, often resulting in speciation and diversification. Parasitic plants range from generalists with many diverse plant hosts to specialists with narrow host ranges. However, it is uncertain whether generalist parasitic plants have preferences for local hosts that have been overlooked in previous field observations and growth experiments. To test for this local adaptation, seeds of a semi-parasitic *Euphrasia* (Orobanchaceae) and three of its common grassland hosts were collected across a wide spatial scale. A common

garden experiment, using thousands of pots, compared fitness and survival across sympatric and allopatric parasite-host combinations. Parasite performance was largely unaffected by host sympatry, suggesting no local adaptation to host populations. However, genetic variation existed between parasite populations in traits such as flower size, and broad preferences for host species were seen. Overall, this project explores the maintenance of a generalist strategy despite potential pressures to adapt to local hosts in these co-evolutionary relationships.

Local adaptation of host-parasite coevolution on spatial networks

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¹ISEM

POSTER SESSION 1 - **P01.195**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Parasites are key components of ecosystems, yet their distribution and diversity across landscapes remain poorly understood. Parasite diversity hot- and cold-spots arise from complex interactions between host populations, ecosystem variables, and environmental changes. To understand coexistence of parasites, we need to understand coexistence of specialist to generalist strategies that is driven by niche breadth evolution in parasites. A key challenge is linking ecological and evolutionary dynamics to the emergence of different properties of modularity or nestedness in host-parasite interaction networks. In this study, we develop a spatially explicit, individual-based simulation model to explore how selection regimes, dispersal, and landscape connectivity shape the emergence and maintenance of parasite diversity and network structure.

Our model considers a gene-for-gene (GFG) framework for infection, incorporating fitness trade-offs associated with resistance and infectivity, as well as virulence-transmission trade-offs. Presence of trade-offs would lead to fluctuating selection dynamics, and without costs would lead to arms race dynamics. We simulate two

landscape structures: Optimal Channel Networks (OCNs), representing aquatic ecosystems, and Random Geometric Graphs (RGGs), representing terrestrial ecosystems. We predict that under fluctuating selection, spatial heterogeneity in adaptation maintains high genetic diversity and drives the formation of modular infection networks, particularly in peripheral or poorly connected patches. In contrast, arms race dynamics lead to directional selection, reduced diversity, and the evolution of nested network structures dominated by generalist parasite genotypes. Landscape topology and the evolution of dispersal behaviors strongly influence these outcomes, modulating the spatial scale of coevolution. Dispersal evolves within a spatial network, allowing us to explore the coevolution of movement strategies and local adaptation.

Our results offer mechanistic insights into how coevolutionary dynamics and landscape structure interact to shape host-parasite networks. By identifying the conditions under which modularity or nestedness arises, this work provides a theoretical foundation for predicting parasite diversity patterns and managing disease risk in complex, changing ecosystems.

Making sense of floral scents: species- and population-level variation in floral scent in *Mimulus* section *Erythranthe* and its evolutionary significance

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POSTER SESSION 1 - **P01.202**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Floral diversity is immense, with nearly 300,000 species of angiosperms described to date. The number of phylogenetically diverse floral species that share common characteristics implies a background pattern of selection acting on floral traits. Unique combinations of these floral traits are hypothesized to reflect selective pressure imposed by certain classes of pollinators. One flower character in particular, scent, has been hypothesized to operate as an unseen signal to attract certain pollinators, particularly when combined with other signals such as color and shape. However, the contribution of scent in pollinator-mediated selection between sister taxa has nearly always been inferred and rarely directly tested, and pollinator sensory mechanisms that drive attraction often remain unclear. The genus *Mimulus* (Phrymaceae) forms a developing model system for studying floral diversity and pollinator-driven speciation using a combination of genetic tools and field ecology. However, the level of floral trait diversity between populations of *Mimulus* section *Erythranthe* has been poorly studied, and most studies have focused on only single inbred lines.

Section *Erythranthe* contains 7-9 species (depending on definition) with potentially

independent transitions from ancestral bee pollination to derived hummingbird pollination, making it an ideal system to study pollinator transitions and population-level variation of floral traits. Populations and species within section *Erythranthe* cluster by floral scent profile despite broad overlap in the volatiles produced, and there is substantial population-level as well as species-level variation in individual floral scent compounds. Surprisingly, the selfing species *M. parishii* produces the most diverse array of floral volatiles of any of the 5 most widespread species. Volatiles across 5 populations of the hummingbird-pollinated *M. cardinalis* differ significantly by population, with qualitative and quantitative floral scent variation normally only seen at the species level. Expression and function of floral scent genes also varies across the section. I will present these results and others from section *Erythranthe* and discuss their implications for species- versus population-level variation, chemotaxonomy, pollinator guild use, ancestral states of floral volatiles, and the role of specific volatiles in biotic interactions. Floral scent may provide a mechanism for diversification and pollinator adaptation in angiosperm taxa, but more studies are needed at multiple taxonomic levels to truly unpack its importance in these processes.

Neuropeptides mediate behavioral and lifespan changes in cestode-infected ants

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POSTER SESSION 1 - **P01.208**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Parasites have evolved strategies to manipulate host behavior to increase their fitness, particularly during intermediate stages of complex life cycles. As Dawkins proposed, such alterations may represent expressions of the parasite's extended phenotype: the host becomes a tool to enhance parasite transmission. Social insects show substantial behavioral variation associated with caste differentiation and division of labor. Neuropeptides—short-chain signaling molecules of 3-100 amino acids—are central regulators of these behaviors, influencing aggression, foraging, stress responses, and task specialization. Synthesized in neurons and acting via specific receptors, neuropeptides are likely targets for parasitic manipulation.

We investigated the role of neuropeptides in the cestode-induced behavioral shifts observed in the ant *Temnothorax nylanderi*, which serves as an intermediate host for the tapeworm *Anomotaenia brevis*. Ants become infected during the larval stage and, subsequently, exhibit extended lifespans and pronounced morphological and behavioral changes. Infected workers are largely inactive, stay near the brood, do not respond to nest disturbances, retain juvenile physiological characteristics, and receive increased care from nestmates. These queen-like traits may enhance predation risk by the definitive woodpecker host, promoting parasite transmission.

To examine the molecular basis of these alterations, we sequenced mRNA from brain and fat body tissues of uninfected workers, uninfected queens, and infected workers. We annotated 2 new neuropeptides—CAPA and an ITG-like peptide—and 17 G protein-coupled receptors in the *T. nylanderi* genome with high tissue-specific expression.

Whole-transcriptome analysis revealed that infected workers exhibit an expression profile intermediate between healthy workers and queens. In the brain, about two-thirds of the neuropeptides were downregulated in infected workers, and a distinct subset showed reduced expression in queens. In the fat body, half of the neuropeptides were similarly downregulated in both infected workers and queens, suggesting that these may be involved in their shared long-lived phenotype.

Interestingly, four neuropeptides with potential roles in the phenotype of infected ants—including low aggression, endocrine reprogramming, and increased desiccation resistance—showed altered expression, suggesting a molecular basis for the behavioral and physiological changes induced by the parasite. Tachykinin and Short Neuropeptide F—linked to aggression and locomotion, respectively—were strongly downregulated in infected worker brains compared to uninfected individuals and queens. Neuroparsin, shown to be a brain-derived juvenile hormone inhibitor in locusts, was upregulated in the fat body of infected ants. Inotocin, a vasopressin-like neuropeptide associated with desiccation resistance through regulation of cuticular hydrocarbon synthesis, was predominantly expressed in the fat body of infected individuals. In clonal raider ants, it further modulates social foraging behavior in an age- and context-dependent manner, contributing to behavioral individuality and the division of labor.

Our findings demonstrate that cestode infection systematically alters neuropeptide signaling in the worker host, partially resembling the profile observed in the long-lived queens. The consistent and specific suppression of neuropeptides in infected individuals, especially in the brain, suggests that this signaling pathway is a key mechanism by which parasites modulate host physiology and behavior to facilitate transmission.

Parasite population genomics sheds light on canid evolution

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POSTER SESSION 1 - P01.201, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Dogs were the first animals domesticated by humans, making them a key focus in evolutionary genomics. While significant progress has been made in tracing the origins of domesticated dogs, the evolutionary histories of pre-domesticated canids and some contemporary wild canid populations, such as the Australian dingo, remain poorly resolved. One approach to help clarify the enigmatic histories of these canids is to analyse the genomes of their parasites. Parasites often share a deep co-evolutionary history with their hosts, and their genomes can serve as useful markers for inferring host origins, migration, and population structure. Since parasite genomes are typically smaller than those of their hosts, they can also be sequenced and analysed more efficiently, requiring less time, lower costs, and fewer computational resources. One of the most important parasites infecting canids is heartworm (*Dirofilaria immitis*), which causes significant morbidity and mortality worldwide. These parasites are widely believed to have dispersed globally with domesticated dogs in contemporary times; however, this hypothesis remains untested. This study aimed to use genome-wide analysis of heartworms to gain insights into the parasite's evolutionary history, as well as that of their canid hosts. **Materials and methods:** We conducted the largest population genomics study of heartworms to date, using whole-genome sequencing data for 127 modern specimens from four continents (Europe, North America, Australia, Asia). Most parasites were obtained from dogs, but some were collected from other hosts such as cats and foxes. The sequencing reads were mapped to a reference genome using a NextFlow mapping pipeline utilising Minimap2, GATK, Samtools, and Sambamba. Variants were identified using GATK and filtered based on various quality metrics. The population structure of the global heartworm dataset was explored using principal

component analysis (PCA), nucleotide diversity (π), absolute nucleotide divergence (DXY), genetic differentiation (FST), and linkage disequilibrium (LD). To assess admixture in the cohort, we analysed ancestry proportions with NGSadmix, produced maximum-likelihood trees using TreeMix, and calculated D-statistics with AdmixTools. Population demographics were investigated by estimating historical population sizes and divergence times of subpopulations using SMC++.

Results: We found distinct genetic differences between heartworms across continents, with divergences (~63 Kya) pre-dating the domestication of dogs (14–40 Kya). This indicates that heartworms have a much older origin and dispersal history than previously recognised, likely involving ancestral wolf or coyote hosts. Our parasite data supports the hypothesis of a once-connected ancient canid population in the Northern Hemisphere that was later fragmented by the formation of large continental ice sheets during glacial periods. These glacial events reduced host connectivity, leading to the deep continental splits we observe in their heartworm parasites today. We also found that Australian heartworms originated in Asia, which is consistent with the migration of dingoes from Asia to Australia thousands of years ago. Finally, the high genetic similarity we found between European and Central American heartworms likely reflects more recent dispersal via domesticated dogs following the colonisation of the Americas in the late 15th century.

Conclusions: Our findings indicate that heartworms have likely been parasitising canid hosts since ancient times, with major dispersal events associated with pre-domesticated canids, dingoes, and more recent domesticated dogs. Most importantly, our study demonstrates the utility of using parasites as markers for inferring the evolutionary history of their hosts.

Questioning the early Miocene origin of grass-feeding noctuids: evidence from a phylogenomic dataset

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POSTER SESSION 1 - P01.229, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Recent studies have indicated that Miocene grassland expansion, driven by global cooling and declining atmospheric temperatures, played a significant role in the radiation of grass-feeding herbivore lineages. The large lepidopteran family Noctuidae, comprising numerous grass-feeding clades, provides a good target taxon for testing these hypotheses. A recently published time-calibrated phylogenetic tree based on a limited number of genetic markers suggested that diversification of the predominantly grass-feeding noctuid tribe Apameini started in the early Miocene, i.e. more than 22 million years ago, potentially coinciding with the expansion of grasslands. However, subsequent analyses using six genes in Sesamiina, a subtribe of Apameini, revealed contrasting macroevolutionary patterns within the insect–grassland system, raising questions about the extent to which grassland expansion influenced the evolution of grass-feeding herbivores. In the present study, we aimed to construct a time-calibrated phylogeny of the tribe Apameini using a large molecular dataset.

Materials and Methods: We first constructed a phylogenetic tree in IQ-Tree using 333 species (296 noctuids and 37 outgroup taxa), based on 548 protein-coding genes totaling 249,138 nucleotides. This tree was then used as a fixed topology for our time-calibrated analysis, which was conducted using a reduced dataset due to software limitations. The reduced data set comprised the 24 longest loci with at least 90% taxon coverage, totaling around 54,000bp. We used the five secondary calibration points taken from recent literature to date the tree in Beast 1.10.4. To detect diversification rate

heterogeneity across the tree, we employed the Bayesian Analysis of Macroevolutionary Mixtures (BAMM) software, version 2.5.0.

Results & Conclusion: The time-calibrated phylogeny inferred in this study indicates that the diversification of Apameini began approximately 8.66 million years ago, i.e. substantially more recently than in previous estimates. Likewise, the divergence between the lineages leading to *Busseola fusca* and *Sesamia nonagrioides* was estimated at around 6.14 million years ago in this study, contrasting with earlier estimates ranging from 18 to 22 million years. Several studies suggest that a major radiation of grasses occurred between 3–9 Mya, driven by environmental changes. Notably, our results place the diversification of Apameini precisely within this time window. Diversification rate analysis using BAMM indicates two instances of rapid radiation in Apameini. One of them, that took place approximately 2 to 5 Mya, involves the genus *Apamea*, which is characterized by larvae feeding largely on the roots of their foodplants. The other rapid radiation event associated with a lineage that comprises several rather diverse genera of the subtribe Apameina, was estimated to have taken place about 3 to 5.5 Mya. Our results prove that the diversification rate of Apameina increased during the period of grassland expansion, though views questioning this pattern had been published earlier. This study thus emphasizes that drastic changes in the Earth's climate can lead to a rapid increase in biodiversity, driven by the accelerated rate of adaptive radiation.

RangeShifter 4.0: modelling spatial eco-evolutionary dynamics for multiple interacting species

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POSTER SESSION 1 - P01.219, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Mitigation of the current biodiversity crisis calls for the improvement of biological forecasts. Both evolution and species interactions have been highlighted as decisive factors determining species' responses to global change but are yet missing from most models projecting these responses. The formulation of accurate predictions is complicated by three factors. First, realistic interactions often involve more than two species. Second, climate change is expected to reshape interaction networks as range shifts bring previously isolated species into sympatry. Third, adaptation to new conditions is likely to alter the intensity or nature of species interactions.

Process-based models are valuable tools to tackle these challenges as they can explicitly integrate the processes underpinning eco-evolutionary dynamics, thus overcoming the limitations of the more popular correlative approaches. The spatially explicit, individual-based model RangeShifter has been used extensively to simulate the eco-evolutionary dynamics of single species, with applications ranging from studies of range expansion of invasive species to theoretical investigations of the interplay between the evolution of dispersal traits and environmental change.

Here, we introduce a new version of RangeShifter that extends the modelling platform to encompass any number of species. The interaction module generalises the Lotka-Volterra models of competition and predation to capture a wide range of interaction types in a single framework. Interactions are defined by a set of coefficients affecting demographic processes such as reproduction and survival. This architecture and the genetic module recently introduced in RangeShifter 3.0 provides the infrastructure to implement evolution of the traits controlling interactions, allowing them to shift along the antagonism-mutualism continuum as environmental conditions change.

Importantly, the flexible simulations enabled by RangeShifter 4.0 will facilitate the development of new theory, for example investigating evolution of species' interactions in changing environments in interplay with demographic and spatial processes, and other traits evolution (e.g. dispersal), and inform empirical applications tackling the interplay between environmental change, biotic interactions and species ranges, beyond single species systems.

Signatures of Reproductive Skew and Corresponding Multiple Merger Coalescence (MMC) in Populations of *Plasmodium falciparum*

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POSTER SESSION 1 - P01.216, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The Kingman coalescent model, which assumes sexual reproduction, discrete, non-overlapping generations, and low offspring variance, does not always apply to species with atypical life histories. One such atypical life history strategy is "sweepstakes reproduction," wherein organisms display considerable variance and skew in individual reproductive output due to complex life cycles and stochastically changing ecological factors, leading to multiple merger coalescent (MMC) genealogies. We hypothesise that *Plasmodium falciparum*, the protozoan malaria parasite, exhibits such reproductive skew and, by extension, MMC genealogies. Analysing genomic data from Southeast Asian *P. falciparum* populations, we observe signatures consistent with MMC: low genetic diversity and a U-shaped synonymous derived allele

frequency spectrum, both deviating from Wright-Fisher-Kingman expectations. Using Sequential Markovian Coalescent (SMC)-based approaches, we infer low to moderate values of the α parameter of the β -coalescent (MMC) model, indicating frequent and strong multiple merger coalescence across the genome. However, validation with biologically realistic simulations reveals that the method shows considerable variability and a slight downward bias in the inferred α estimates. These findings support the presence of MMC in *P. falciparum*, challenging the validity of traditional population genetic analyses, while also highlighting the need for further research to strengthen confidence in our insights into the strength and mechanisms driving MMC in this malarial parasite.

Syntopy promotes song divergence in a Neotropical avian radiation

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*POSTER SESSION 1 - **P01.191**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM*

Theory predicts that selection against maladaptive hybridization leads to divergence of sexual characters in co-occurring closely related species. Consequently, signal disparity should be greater between sympatric versus allopatric lineage pairs. However, this pattern may also result from species sorting or the greater evolutionary age of sympatric pairs. We used species pairs comparisons to examine the existence of acoustic divergence in a Neotropical montane radiation, the Rhinocryptidae, whose members tend to occupy different elevational ranges. Most rhinocryptids exhibit conservative morphology and are only differentiated by song attributes. Our results show that sympatric species pairs that overlap in elevation exhibited overall greater song divergence compared to

allopatric species pairs after controlling for morphological differences, age and phylogenetic effects. Song divergence decreased when excluding sympatric pairs that do not overlap in elevation, suggesting that selection for improved species identification between co-occurring (syntopic) species accentuates signal differentiation. Comparative evolutionary models of signal differentiation over time revealed a similar pattern which suggests that sexual selection in syntopy might have driven reproductive character displacement in this radiation. We conclude that selection against the production of unfit hybrids could favor acoustic traits that reliably signal species identity in tropical environments where many taxa are poorly differentiated by visual attributes.

The genomic basis of an obligate ant-plant symbioses

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*POSTER SESSION 1 - **P01.205**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM*

Mutualisms are ubiquitous across taxa, playing a pivotal role in the evolution and functioning of life on earth. These interactions vary from loose, facultative interactions across many species to tight, one-to-one interactions on which species depend for survival or reproduction. How these interactions impact the genome evolution of interacting individuals has largely only been explored in endosymbionts with 'macro' mutualisms rarely studied. We address this question using the farming symbiosis between six species of *Squamellaria* plants and their obligately-specialised cultivator *Philidris nagasau*. This provides a great study system as three other *Squamellaria* species facultatively associate with up to 14 other species of arboreal ants allowing for direct comparisons. Additionally, the ant genus *Philidris* includes obligate, facultative and non-plant

mutualists thus this system offers a range of dependencies in both macro-mutualists. We Used high coverage PacBio Hifi, Hi-C and illumina sequence data to assemble, annotate and compare key genome characteristics between the obligate and facultative species and resolve their respective phylogenies. We then utilise comparative genomics to trace the evolution of interaction-related genes among obligate and facultative *Squamellaria*, *P. nagasau*, and other facultatively associating *Philidris* ant species, revealing dependency specific patterns in gene family evolution. Combined with existing ecological observations, these results reveal how mutualistic dependency influences genome evolution, establishing a foundation for understanding the genomic basis of complex, non-microbial mutualisms.

The INTERACT project: Understanding evolution of common bean plant-plant interactions

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POSTER SESSION 1 - P01.213, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Agroecosystems have undergone significant transformations due to crop domestication, modern breeding, and intensive agricultural practices. These changes have resulted in increased environmental control and reduced genetic diversity of cultivated stands, greatly simplifying abiotic and biotic interactions. Among biotic interactions, sustainable strategies based on positive plant-plant interactions should be promoted to address challenges posed by climate change. However, their genetic and molecular bases remain poorly understood, highlighting a critical knowledge gap that must be filled.

Within this framework, the INTERACT project aims to unravel key regulatory pathways involved in intraspecific plant-plant interactions by analyzing gene expression, metabolite profiles, and microbial communities across wild, landrace, and cultivated genotypes of common bean (*Phaseolus vulgaris*), grown in binary mixtures and pure stands. The species complex evolutionary history, shaped by two independent domestication events in geographically distinct gene pools (Mesoamerican and Andean), provides a unique replicated system for studying these mechanisms.

Materials and Methods: This study uses 18 common bean genotypes (9 for each of the Mesoamerican and Andean gene pool), representing the two stages of crop evolution (wild --> landraces; landraces --> cultivars), resulting in 3 wild, 3 landraces, and 3 modern cultivars for each gene pool. Genotypes were selected based on available genotypic data from previous projects (INCREASE, BEAN_ADAPT, BRESOV, NextBEAN), ensuring a wide range of genetic backgrounds.

The experiment was conceived to test 90 genotype combinations (including 18 pure stands) across both gene pools arranged in a complete randomized block design (CRBD) with three replicates. These include 45 combinations

per gene pool: 9 pure stands and 36 mixed pairs (wild-landrace, wild-cultivar, and landrace-cultivar). Pots were filled with a uniform soil mix from a local organic farm, vermiculite, and universal soil.

Two parallel experiments (destructive and non-destructive) have been performed in controlled environment. In the first one, root and leaf tissues have been collected after four weeks for RNA extraction (transcriptomics) and metabolomics analysis. Soil samples have been obtained for DNA extraction (metagenomics). For metabolomics and transcriptomics both pairs of the mixtures were sampled separately, while for metagenomics a composite sample of the two pairs was collected. The non-destructive experiment is currently ongoing; it is focused on seed production for metabolomics. Seeds will be also analyzed using a multispectral camera to capture images across multiple wavelengths.

Results: Expected results aim to identify differentially expressed genes associated with intraspecific plant-plant interactions. Metabolomic analysis will uncover changes in chemical signaling, while microbial community profiling will provide insights into how these interactions are influenced by soil microbiota. Comparative analyses between the Mesoamerican and Andean gene pools will assess the effects of domestication and breeding.

Conclusions: This integrative study will enhance our understanding of the agroecological genomics foundations of intraspecific interactions in common bean. By analyzing genes, transcripts, metabolites, and microbial communities, we will uncover how domestication and breeding have shaped plant communication mechanisms. The findings will contribute to plant breeding programs aimed at improving crop resilience, productivity, and sustainability by leveraging positive plant-plant interactions.

Towards evidence for plant-bacteria coevolution: an ecophylogenetic framework reveals selection through niche construction

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POSTER SESSION 1 - **P01.198**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Biotic interactions are responsible for major evolutionary changes across multiple spatial and temporal scales.. One of the most intricate and complex biotic interaction is the association between plants and microorganisms that may have originated around 460-475 Mya. The extent to which coevolution may drive natural selection and shape angiosperm diversity is still debated, particularly because no unequivocal evidence has been provided regarding reciprocal evolutionary changes. Understanding if plant-driven niche construction led to phylogenetically structured microbial communities is crucial, as it can reveal how plants selectively and consistently shape their associated microbiota through space and time, offering an opportunity for identifying coevolutionary dynamics between plants and their microbiota. The concept of coevolution is still subject to multiple debates and, apart from semantic questions, reflects the need to develop experimental approaches which take into account the complexity of biotic interactions. Despite the known benefits of plant microbiota on growth and nutrition, the mechanisms of association remain largely unknown whereas they do constitute the basis and requirements for coevolution to occur. We used a spatio-temporal experimental approach under controlled conditions where we grew 108 *Pisum* spp.

(Fabaceae; Fabeae) accessions and investigated the composition and structure of bacterial communities in distinct plant-associated microhabitats (rhizosphere, rhizoplane and endosphere) at different growth stages (vegetative, flowering and seed filling). Using full-length 16S rRNA gene sequencing, we inferred a phylogeny for 1604 ASVs present in a bulk soil and analyzed their relative abundances across a spatial (i.e. plant-associated microhabitats) and temporal (i.e. plant growth stages) gradient. We found that temporal and spatial changes in the composition and structure of bacterial communities were coupled with shifts in phylogenetic diversity and followed both over- and underdispersion models along a spatial gradient. Our findings suggest that plants play a role in driving the evolution of bacterial communities through niche construction. We argue that plant-microbiota interactions illustrate pairwise coevolution, where both plants and bacteria act as active niche constructors, shaping their shared environment and adapting to the changes they induce. We will discuss how our ecophylogenetic approach can be used to explore plant-microorganisms' interactions to disentangle the mechanisms (i.e. traits under reciprocal selection) and dynamics (i.e. plant-bacteria phylogenetic correlations) shaping these associations in light of coevolutionary processes.

Transcriptomic signatures of infection and insights into transgenerational immune priming in an invertebrate host

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*POSTER SESSION 1 - **P01.204**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM*

Parasites impose substantial fitness costs on their hosts, often triggering complex immune and life-history responses. Recent evidence suggests that invertebrates, despite lacking a sophisticated adaptive immune system like vertebrates, can influence the immune response of their offspring and of subsequent generations, a phenomenon known as immune priming. However, the molecular mechanisms underlying both within- and transgenerational immune response remain largely unexplored, particularly in non-insect invertebrates.

Using the *Daphnia magna*–*Metschnikowia bicuspidata* host-parasite system, we first investigated within-generational transcriptomic and phenotypic responses to infection. Exposed-uninfected individuals upregulated genes associated with haemocyte recruitment and gut epithelium reinforcement, suggesting that enhancing structural barriers may contribute to parasite resistance. These individuals also delayed reproductive maturation, likely reflecting a cost of immune activation, but maintained reproductive output over time. Thus, overall fecundity was compensated later in life. In contrast, infected individuals exhibited metabolic shifts consistent with host

exhaustion, aligning with the terminal investment hypothesis, whereby resources are reallocated from somatic maintenance to immediate reproduction under high mortality risk.

In transgenerational comparisons, both maternal and grandmaternal immune priming resulted in upregulation of genes encoding RNA polymerase subunits, suggesting an enhanced transcriptional activity as a conserved signature of immune priming. However, grandmaternal priming induced a significant downregulation of genes involved in DNA replication, repair, and proteolysis, suggesting a more extensive regulatory shift. The increased number of differentially expressed genes in grandmaternal priming compared to maternal aligns with previous findings in *Daphnia* that transcriptional responses to environmental cues can intensify across generations.

Our findings provide novel insights into the molecular and physiological mechanisms of invertebrate immune responses, shedding light on both within- and transgenerational strategies that shape host responses to parasitic infection.

Twenty-years of Tuberculosis-driven selection on meerkat MHC

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POSTER SESSION 1 - P01.224, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Pathogen-mediated balancing selection (PMBS) drives host evolution across the tree of life. Distinguishing between the three main mechanisms underlying PMBS, i.e., rare-allele advantage, fluctuating selection and heterozygote advantage, remains difficult, limiting our understanding of frequency-dependent adaptations by hosts and counter adaptation by pathogens. Here, we leverage immune genetic and disease surveillance data from over 1,500 wild meerkats (*Suricata suricatta*) to track how selection by the tuberculosis (TB)-causing *Mycobacterium suricattae* shaped the evolution of the Major Histocompatibility Complex (MHC) over two decades. Compared with neutral genetic markers, we detect more rapid differentiation and recycling of alleles at the MHC-DRB loci, suggesting that TB imposes

strong PMBS on wild meerkats. In addition, we show that meerkats carrying the MHC allele Susu-DRB*13 were initially more likely to be infected with TB, with the effect reversing over the course of the study, followed by an increase in the frequency of Susu-DRB*13. Meerkats carrying Susu-DRB*13 also showed slower progression to TB symptoms and longer survival once clinical signs of TB appeared. Life-time reproductive success reflected the resistance effect conferred by Susu-DRB*13. Based on several lines of evidence, we propose that the recycling of a rare-allele or fluctuating selection, rather than heterozygote advantage, drive our observation in this longitudinally sampled wild mammal population.

Unraveling the molecular basis of host plant adaptation in a generalist herbivore

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POSTER SESSION 1 - P01.220, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The antagonistic interactions between plants and herbivores promote evolutionary change. Studying the evolution of herbivore adaptation to different host plant species is a fundamental cornerstone of evolutionary biology with clear applications for sustainable pest management. Generalist herbivores, which can colonise multiple host plant species are of particular interest, as hybridization between genetically distinct populations can increase the standing genetic variation and therefore the adaptive potential of the species. To identify the genetic basis of host plant adaptation we focus on *Tetranychus urticae*, a generalist mite known for its adaptive potential, which is evidenced in its immense host range and ability to develop metabolic resistance to xenobiotics. Despite its widespread distribution and detrimental effect to crops, the molecular underpinnings associated with the potential of host adaptation and the consequences of host adaptation in this, and many other pests remain elusive. Here, we conduct two experimental approaches in

parallel, to identify and map the genetic basis of host plant performance and adaptation in genetically distinct populations of *T. urticae*. We create a genetically diverse mite population in the laboratory, subject it to selection using a challenging plant for spider mites, tomato, and map genomic regions linked to the phenotypic evolution of increased reproductive performance. In parallel, we map genomic regions responsible for performance on tomato segregating in field mite populations, by comparing the genomes of pooled individuals from an F2 backcross between populations with high and low reproductive performance on tomato. Both approaches revealed specific and shared genomic regions associated with host plant performance and adaptation and key candidate genes were identified. Our findings highlight the power of spider mite genetic approaches to identify the complex genetic basis of host adaptation in generalist herbivores.

When Durum Wheat grows together with Alfalfa: The Genetic Dialogue of Neighboring Plants

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POSTER SESSION 1 - **P01.226**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Growing environmental constraints and the need for more sustainable food systems promote crop diversification and the harnessing of ecological processes to enhance resilience and reduce external inputs. Among the promising strategies, intercropping—growing multiple species together—optimizes resource use and fosters beneficial plant-plant interactions (Cougnon et al., 2022). However, designing productive and stable species mixtures requires both careful management of within-species variability and a better understanding of the genetic mechanisms underlying such interactions.

Plant-plant interactions are major drivers of individual performance and community outcomes. In crop mixtures, they include both intra- and interspecific processes that shape competition, facilitation, and resource allocation. While their ecological consequences have been widely studied (Becker et al., 2023), their genetic basis—and especially their potential to evolve—remains underexplored. We address this gap using a model intercrop composed of *Triticum durum* (wheat), an annual income species expected to enrich grain protein content, and *Medicago sativa* (alfalfa), a perennial service species contributing to weed control and nitrogen input. This cereal-legume combination offers a compelling system to investigate interactions between species with complementary functions and life histories.

To dissect these interactions, we rely on an individual-centered experimental design, where focal wheat plants are surrounded by variable neighbors from both species. This layout captures the intra- and interspecific genetic context in which each individual develops. By manipulating the genetic composition of neighbors, we analyze how their identity and diversity shape the phenotype of focal plants.

We apply a quantitative genetic model estimating direct genetic effects (DGEs)—due to an individual's own genotype—and indirect genetic

effects (IGEs)—due to the genotypes of surrounding individuals (Bijma et al., 2007). Our model extends these concepts across species boundaries, capturing how a wheat genotype affects neighboring wheat and alfalfa plants, and vice versa. This is especially relevant in cereal-legume systems, where traits like nitrogen fixation or canopy architecture drive strong facilitative interactions.

By partitioning phenotypic variance into intra- and interspecific genetic components and estimating covariances among them, we gain insights into how selection may act not only on individual performance but also on the ability to influence or respond to neighbors. Across functional traits (e.g., tiller number, plant height, leaf nitrogen), agronomic traits (e.g., biomass, yield components), and spectral data (NIRS of leaves and grains), we show that phenotypes are shaped by both self-genotype and the genotype of multiple neighbors.

We demonstrate the existence of heritable interactions at both intra- and interspecific levels. For wheat, we estimate pseudo design heritabilities for DGEs and IGEs of 0.57 and 0.29 (plant height), and 0.52 and 0.26 (leaf nitrogen), confirming the capacity of our design to detect both. Simulations suggest these effects can evolve over time, shaping trait trajectories and breeding outcomes.

By formalizing multispecies genetic interactions in an individual-centered framework, our work bridges quantitative genetics, evolutionary biology, and agroecology. It offers a novel lens to study cooperation and competition in crop mixtures and a practical toolbox for their design. This approach opens new perspectives: in breeding, by accounting for neighbor effects in both pure and mixed stands; in evolutionary biology, by exploring how interspecific interactions drive trait coevolution; and in agroecology, by illuminating the mechanisms behind facilitation and complementarity.

Zostera marina and Labyrinthula spp.: Exploring Pathogen Virulence and Host Defense Across A Thermal Gradient

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POSTER SESSION 1 - P01.218, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Seagrasses are a unique group of marine flowering plants that comprise 72 species forming large meadows in coastal waters around the globe, except Antarctica. By providing various ecosystem services, such as carbon sequestering, coastal protection, and biodiversity enhancement, seagrass meadows are among the most productive ecosystems. Despite their ecological importance, seagrass ecosystems are experiencing widespread and accelerating declines. Losses are driven by a combination of anthropogenic and natural stressors, including eutrophication and direct habitat destruction. Natural threats such as eelgrass wasting disease, caused by the causative agent *Labyrinthula zosterae*, have only recently come into focus. This protist is ubiquitous in seagrass meadows of various species and can induce necrosis and ultimately death in affected plants, while it is often asymptomatic. The most prominent virulent outbreak occurred at the Western Atlantic Coast in the 1930's diminishing the abundance of eelgrass to 10% of the initial population.

Previous studies involving the temperate seagrass species, *Zostera marina*, inoculated with *Labyrinthula* spp. have demonstrated highly dynamic host-pathogen-environment interactions that cannot be readily explained and appear to shift in response to changing environmental conditions. To further investigate these dynamics, we will employ a temperature gradient-based approach (ranging from 20-30°C) to identify a thermal threshold at which the Eelgrass-*Labyrinthula* interaction may shift from a non-pathogenic to a pathogenic relationship, thereby affecting the performance of both host and pathogen. Seeds of Baltic *Zostera marina* were surface-sterilized to reduce the likelihood of pre-existing *Labyrinthula* contamination and subsequently

planted in climate-controlled chambers. Additionally, Mediterranean seedlings were grown from non-surface sterilized seeds. Moreover, two *Labyrinthula* spp. strains were isolated from Baltic and Mediterranean eelgrass plants and cultivated on seawater-agar medium plates. Strains and plants from two different bioregions will be used since localized historical outbreaks may have driven host populations to evolve mechanisms of tolerance to *Labyrinthula* infection. Moreover, not all *Labyrinthula* strains exhibit virulence toward host populations, potentially due to specific host and pathogen genotype interactions, that formed in co-evolution and local adaptation. These processes could be disrupted by changes in biotic and abiotic conditions. Baltic and Mediterranean eelgrass plants will be inoculated in a crossed design with both *Labyrinthula* strains and exposed to a range of 11 different temperatures representing benign to extreme temperature stress over a period of three weeks, including a recovery phase. Inoculation success will be quantified using qPCR to determine the abundance of *Labyrinthula* cells on eelgrass tissues. In addition, host and pathogen performance will be evaluated through gene expression patterns, morphometric parameters including plant survival and growth rates, and stress indicators such as photosynthetic and respiratory rates. To date, we have successfully isolated multiple *Labyrinthula* strains from both the Baltic and Mediterranean Seas and have begun preliminary inoculation trials. Our upcoming results will provide valuable insights into species-specific host-pathogen interactions and how these are modulated by temperature, thereby advancing our knowledge of the mechanisms driving seagrass wasting disease.

Bacteria-phage coevolution drives patchy distribution of plant disease through phage resistance-virulence trade-offs

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MEETING ROOM 113, August 18, 2025, 10:30 AM - 12:30 PM

Bacteria-phage coevolution is pervasive in nature, affecting the global nutrient cycling and the composition and functioning of microbial communities. While reciprocal adaptations often have correlated fitness effects on coevolving species, it is unclear how these changes cascade in food webs, affecting the ecology of the surrounding communities. Here, we used a combination of field, greenhouse and lab experiments to link coevolutionary patterns between phytopathogenic *Ralstonia pseudosolanacearum* bacterium and its phage parasites to bacterial wilt plant disease patterns in tomato fields. By sampling four geographically disconnected fields, we show that bacteria and phages are locally adapted between and within fields, where phages had the highest infectivity on

sympatric bacteria and bacteria showed the highest resistance when isolated from the healthy versus diseased plants. The modularity of phage-bacteria coevolution could be explained by field-specific anti-phage defense system patterns and direct experiments where phages selected for field-specific mutations in different phage receptor genes. Crucially, phage resistance showed strong antagonistic pleiotropy with virulence measured in planta, explaining why phage-resistant but weakly virulent pathogen isolates were associated with healthy tomato plants within fields. Our findings demonstrate that bacteria-phage coevolution can cascade through trophic networks, driving a patchy plant disease distribution through phage resistance-virulence trade-offs.

Changing the script: ecological reversal of predator–prey roles in microbial communities

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Ecological variation influences the character of many biotic interactions, but examples of predator–prey reversal mediated by abiotic context remain rare. We show that the temperature at which prey are grown before interacting with a bacterial predator can determine the very direction of predation, reversing predator and prey identities. While *Pseudomonas fluorescens* reared at 32°C was extensively killed by the generalist predator *Myxococcus xanthus*, *P. fluorescens* reared at 22°C became the predator, slaughtering *M. xanthus* to extinction and growing on its remains. This reversal was not limited to a single species pair: diffusible molecules produced by *P. fluorescens* also killed two other

phylogenetically distant bacterial species, among several examined.

Our results suggest that predator and prey identities may not be intrinsic properties of species, but rather context-dependent roles that can shift with environmental conditions. They also suggest that a larger proportion of microbial warfare results in predation—the killing and consumption of organisms—than is generally recognized.

More broadly, our findings support the view that biotic interactions move along dynamic continuums rather than occupy rigid categories, and highlight how integrating ecological variability into evolutionary frameworks could open exciting ways to look at natural communities and their responses to rapid environmental change.

Co-circulation and co-infection: parasite evolutionary ecology across scales

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Multiple parasites strain and/or species often co-circulate simultaneously in the same host population or even co-infect the same host individuals. Each process – co-infection and co-circulation, respectively – can lead to interactions between parasites. For example, parasite can interfere with each other directly within the same host individual, or indirectly by removing susceptible hosts from the population. However, co-infection and co-circulation are often investigated by different fields (e.g., immunology versus epidemiology). This has resulted in a fundamental gap in our understanding of how parasite interactions at these two levels affect each other, and on the long-term evolutionary implications.

I will present a framework aimed at bridging between individual- and population-level perspectives of interactions among parasites, developed by a consortium of evolutionary biologists and ecologists investigating the topic from theoretical, experimental and field perspectives. First, we employed the Susceptible-Infected framework to characterise the potential range of individual- and population-level host interactions that may occur among co-circulating parasites. Second, we mapped

empirical examples from the literature onto this framework, to illustrate the mechanisms driving both types of interactions across diverse host-parasite systems and the evolutionary consequences. I will also briefly present results from an ongoing project, combining experimental investigations of co-infection and co-circulation dynamics in an aquatic model system with mathematical modelling.

Careful modelling and experiments can help disentangling when interactions at one scale affect those at another, and how ascribing a pattern to a within-host mechanism that is in fact driven by a between-host process (or vice versa) can be misleading. Given the steady rise of emerging and re-emerging infectious diseases, novel parasite combinations are increasingly likely to be brought together, resulting in new and more frequent interactions and opportunities for selection and evolutionary changes to occur. Thus, better understanding of general rules for interactions between parasites, and key sources of variation in outcomes, will provide insight for public health, conservation and agriculture, all of which is all the more important in the current context of rapid global change.

Coinfection with a both a competitor and a facilitator parasite increases within-host growth rate and transmission

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Parasite-parasite interactions are predicted to impact parasite life-history evolution, including virulence and transmission. For instance, competition among parasites for shared host resources or mediated via the immune system can select for higher virulence and transmission. There may also be positive interactions among parasites, but less is known about how facilitation might impact parasite evolution.

This experiment investigates how evolution of the spider mite *Tetranychus evansi* in coinfection with a competitor, another spider mite *T. urticae*, or a facilitator, Tomato spotted wilt virus (TSWV), impacts life-history evolution on tomato plants. *T. evansi* competes with *T. urticae* for shared host resources and via the plant immune system, whereas TSWV has a positive effect on *T. evansi* life-history traits, possibly via negative immune cross talk. We used experimental evolution to evolve *T. evansi* on tomato plants in 1. coinfection with *T. urticae*, 2. coinfection with TSWV, or 3. in single infections over > 18 generations.

Following, 18 generations we assessed how *T. evansi* life-history traits (including virulence and transmission) evolved in the different coinfection treatments on leaf discs and whole plants. We are currently extracting virulence traits.

On leaf discs, *T. evansi* evolved in coinfection with either TSWV or *T. urticae* produced a

higher number of adult daughters and had earlier transmission than *T. evansi* that evolved in single infection at low densities. Similarly, on whole plants preliminary results suggest that transmission is higher for *T. evansi* lines that evolved in coinfection with TSWV or *T. urticae*.

Thus, as predicted, evolving in coinfections with a competitor increases within-host growth and transmission. It is not immediately clear why evolution under facilitation with TSWV had the same effect, but it is possible that TSWV increases intraspecific competition, both directly due to higher growth rates and indirectly by reducing host plant size and thus available resources.

When in coinfection with *T. urticae*, or at higher *T. evansi* densities, there was no difference due to evolution regime (single or coinfection) in offspring production or transmission, showing that evolving with a competitor parasite did not increase competitive ability. In contrast, *T. evansi* evolved in coinfection with TSWV had a weaker effect on *T. urticae*, enabling them to produce more offspring that became adult. The reason for this is not entirely clear, but this result does show that evolving in coinfection with one parasite (TSWV) can change the outcomes of coinfection for another parasite (*T. urticae*).

Competition and facilitation at the within- and between-host levels between spider mites evolving in metal-accumulating plants

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Evolution can alter coinfection dynamics by modifying traits involved in transmission between hosts, a possibility that has been poorly studied, especially in a heterogeneous host population. Here, we test how evolution of ectoparasitic spider mites on tomato host plants affects their transmission in different scenarios. To do so, we performed experimental evolution of *Tetranychus urticae* or *T. evansi* on tomato plants that accumulated cadmium or not. Then, we measured how evolution affected transmission and host choice in presence or absence of competitors and population growth rates in competition in a heterogeneous environment. We did not detect signs of adaptation to that heterogeneous environment in the *T. urticae*

populations. However, *T. evansi* populations evolving in cadmium had higher growth rates in that environment than control populations. Moreover, when placed together on leaves with cadmium, both species dispersed more than from leaves with no cadmium, but only when the competitor evolved in cadmium. Similarly, when together, both species increased their preference toward leaves with cadmium, but only when *T. evansi* evolved in cadmium. This suggests that evolution within a host modifies traits that are expressed between different types of hosts. Hence, evolution can shape coinfection dynamics of two species by modifying traits that affect transmission dynamics between hosts.

Costs of immunity avoided by immune suppression in novel terminal investment response

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Immunity is not only costly for the host but can drive an arms race stimulating the evolution of lethal microbes. However, eco-immunity research has not established whether animals have evolved different mechanisms to manage common infections or determined whether host responses could, in fact, drive the evolution of mutualisms. Here, we show that insects do not fight common low-dose topical infections with immunological mechanisms that improve post-infection survival; instead, they employ terminal investment mechanisms that enhance reproduction with immediate survival costs. We focus on when, how, and why insects adopt terminal investment and how these responses contribute to the evolution of mutualisms. We demonstrate that the fruit fly, *Drosophila melanogaster*, responds to low-dose topical exposures of endemic fungi by succumbing to infection via low-cost terminal

investment, while combating high-dose infections with reproductively detrimental immune responses. Our discovery of a low-cost reproductive strategy mediated by Turandot C (TotC) represents the first non-immunological genetic mechanism. The contrasting fitness effects of the expression of TotC and the canonical immune gene Df show that terminal investment operates through stress-responsive pathways divergent from innate immunity. And, we show how recent mathematical modelling illuminates how life history responses can contribute to the evolution of host-microbe mutualisms. Our study is important because it shows how evolution of immune suppression in the host can be cost-effective for the host. It identifies key fitness trade-offs that limit the evolution of defence against infection. It suggests that variation in how hosts respond to infection contributes to the antagonism / mutualism continuum.

Evolution of buzz pollinated flowers in response to pollen-feeding insects: Pollen dosing in response to vibrations

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Although many plant species use nectar and other resources to attract and reward floral visitors, thousands of nectarless species rely on pollen as the main reward. By consuming pollen, which in this case function as both the carriers of the plant's male gametes and food for visitors, floral visitors can impose conflicting selective pressures on pollen removal. Previous work has shown that successful pollen export to other plants is a decelerating function of the amount removed per visit. Thus, removing more pollen per visit does not necessarily translate to more pollen exported. Plants have therefore evolved strategies that allow them to control the amount of pollen removed per visit, i.e., "pollen dispensing" strategies. About 20,000 species of flowering plants have evolved specialised flowers that conceal pollen inside tubular structures, usually anthers, that open only through small pores at the tip. These tube-like or poricidal anthers potentially control pollen dispensing. Yet, some bee species have evolved the capacity to produce high-power

vibrations that shake pollen out of poricidal anthers and could overcome this dispensing mechanism. Here we present the results of experimental studies assessing pollen release during floral visits involving vibrations (buzz pollination). Specifically, we assess how the type of pollination vibrations affects the amount and rate of pollen released. To do this we use a combination of experiments with bumblebees and with mechanical shakers where we can mimic vibrations produced by different types of bees on flowers of the genus *Solanum*. Our results suggest that poricidal flowers strongly limit pollen release during single visits suggesting that they work as pollen dispensing mechanisms. Our findings suggest that in addition to the physical constrain of pollen release by the evolution of small anther openings, other developmental processes are involved in limiting the amount of pollen that bees can removed from buzz pollinated flowers during individual visits.

Evolution of the plasticity of pathogen virulence in temporally and spatially variable environments

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Some pathogens have evolved the ability to plastically adjust their infection strategies to cope with the variability of their environment. Yet the ecological conditions promoting the evolution of pathogen plasticity remain unclear. Here we analyse the evolution of a pathogen with the ability to perceive the infection status of neighbouring hosts and we explore when this information may favour the evolution of plastic virulence strategies. First, we show that virulence plasticity does not evolve when the environment is constant. Second, we show that temporal or spatial fluctuations of the environment can promote the evolution of lower virulence in informed pathogens because the signal

perceived by the pathogen provides an indirect information on the change of the quality of the environment. This indirect information takes the form of a negative covariance between the perception of the signal and the availability of susceptible hosts. We explore and contrast the influence of temporal and spatial structure on the evolution of plasticity. This work clarifies the conditions promoting the evolution of phage communication systems such as Arbitrium. More generally, this analysis provides a general theoretical framework to understand the adaptive nature of communication and plastic life-history strategies of microbes in variable environments.

From mosquitoes to cuckoo bumblebees; selfish genetic elements to spotted hyenas; penguins to pathogens: can we explain how antagonists choose their victim?

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The way in which antagonists choose their victim is fundamental to antagonistic interactions. In this talk, I will argue that a trade-off between victim quality and vulnerability generalises across interactions – from klepto-parasites, selfish genetic elements, host-consuming parasites and brood parasites, to pathogens, predators and herbivores.

This framework defines quality as the value of resources a host has, and vulnerability as the ease with which a parasite can obtain those resources. Parasite ‘choice’ can refer to flexible decision-making or to specialisation over evolutionary time. A parasite can choose a low-quality host, which is easier to attack but offers limited resources; or a high-quality host, which is more challenging to attack but offers more resources if the parasite is successful. For example, louse flies can take larger blood meals from chicks in good body condition, but face weaker immune defences from chicks in poor body condition (Bize et al., 2008). Similarly, klepto-parasitic gulls obtain higher-value food items from adult pelicans, but can more easily steal food from juvenile pelicans (Carroll & Cramer 1985).

The optimal decision for a parasite will depend on its ecology and the shape of the trade-off in a given system. I will first discuss the conditions required for the trade-off to apply. Many studies of different types of parasitism and predation across taxa have

investigated traits pertaining to quality or vulnerability, but their findings have not previously been integrated. Doing so makes it possible to draw out broad principles that determine whether quality or vulnerability has the greater impact on host choice. For example, vulnerability becomes more important when parasites manipulate host quality post-infection (e.g. via parasitic castration).

This approach can help resolve contradictory findings, such as why the same parasites choose high-quality hosts in some studies, and low-quality hosts in others. It can be used to project the effects of global change on host-parasite and predator-prey dynamics. Factors which directly affect host quality, such as food availability and the presence of toxins, are currently experiencing dramatic shifts due to climate and other sources of anthropogenic change. This predicts shifts in the optimal victim based on the trade-off between quality and vulnerability.

The framework also creates an integrated perspective on coevolutionary adaptations. Across systems, hosts evolve lower vulnerability – or, rarely, lower quality (e.g. bacteria depleting their own deoxynucleotides in response to phage infection). Hosts can manipulate parasite choice by deceptively signalling low quality (e.g. thanatosis, masquerade) and low vulnerability (e.g. Batesian mimicry).

I'm the captain now: Host fitness is predicted by host life-history strategy in an emerging model host-parasite system

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MEETING ROOM 113, August 18, 2025, 2:00 PM - 5:00 PM

Parasitic manipulation of hosts can alter resource allocation, impacting both individual fitness and population dynamics. Zooplankton, the first animal link in many aquatic food webs, are hosts to thousands of parasites distributed globally, yet the consequences of their infections remain understudied. This gap is especially critical given their role as first intermediate hosts for parasites involved in Neglected Tropical Diseases (NTDs), where unresolved early-stage dynamics complicate disease control. In trophically transmitted parasites, where hosts do not recover from infection, life-history theory predicts increased reproductive effort following exposure and reduced fecundity after infection due to resource depletion. Using an emerging model system of the cyclopoid copepod *Acanthocyclops robustus* and the trophically-transmitted tapeworm *Schistocephalus solidus*, we tested these predictions in controlled laboratory settings. We find that exposed (but uninfected) females amplify their reproductive effort: they produce larger and more frequent

clutches, consistent with adaptive reallocation toward reproduction under infection threat. In contrast, infected females become sterile, ceasing reproduction despite prior mating and known sperm storage capacity. Parallel experiments testing resource scarce conditions suggest that sterility is not explained by energetic limitation alone, positioning *S. solidus* the culprit for the fecundity loss in copepod hosts. These results offer empirical support for theoretical models predicting strategic reproductive shifts in hosts prior to infection and sterility post-infection in response to resource-depleting parasites. By disrupting copepod fecundity, a high prevalence of infected individuals may alter zooplankton population dynamics. Shifts in primary consumer population structure have implications for both ecosystem stability and the availability of suitable hosts for parasites, both of which are critical for ecological forecasting and the control of NTDs.

Landscape structure as a driver of eco-evolution in host-parasite systems

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Spatial network structure of biological systems drives ecology and evolution by distributing organisms and their genes. The ubiquitous host-parasite systems are no exception. However, past theoretical work has largely focused on simple spatial structures, such as grids, hampering the translation of theoretical predictions to real ecosystems. Thus, we develop an eco-evolutionary metapopulation model of host-parasite dynamics where hosts and parasites disperse through realistically complex spatial networks representing major biomes using river-like and terrestrial-like networks. We generate the testable

prediction that parasite virulence, or how parasites harm their hosts, peaks at intermediate dispersal values in river-like systems while it increases with increasing dispersal in terrestrial-like systems. In river-like systems, virulence also reaches higher overall values. Moreover, we explore the evolution of further interaction traits, such as dispersal and resistance in a 3-dimensional trait space. Our study highlights how eco-evolutionary feedbacks can be understood in light of spatial network structure by linking network topology to classical evolutionary mechanisms such as kin selection.

Macroevolutionary Patterns in Trees Shaped by Three Key Mutualisms: Pollination, Seed Dispersal, and Mycorrhizal Symbiosis

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Mutualistic interactions such as mycorrhizal symbiosis, seed dispersal, and pollination are key drivers of plant diversification in terrestrial ecosystems. Yet, we still lack a clear understanding of how these interactions have evolved together to shape global patterns of tree diversity. In this study, we analyzed the joint evolutionary patterns of mycorrhizal type, seed dispersal mode, and pollination mode using two global databases: one with 699 tree species based on direct field observations of mycorrhizae, and another with over 10,000 species based on phylogenetic inference. Despite differences in data certainty and coverage, both datasets showed consistent results.

We found strong evidence of co-evolution among the three traits. Arbuscular mycorrhizal (AM) trees are typically associated with biotic seed dispersal and pollination, which promote long-distance gene flow. In contrast, ectomycorrhizal (EM) trees are more likely to have wind-driven seed and pollen dispersal, linked to shorter-range processes. Our findings suggest that mutualistic syndromes involving belowground and aboveground partners have evolved in concert and have played a central role in the macroevolutionary diversification of trees across ecosystems worldwide.

Repeated breakdowns of mutualism into parasitism and expansions of ecological opportunity in a globally distributed plant-insect symbiosis

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Background: Although mutualism is commonly thought to be inherently unstable because of the opportunity for selection to favor antagonism by interacting partners, few macroevolutionary transitions from mutualism to parasitism have been reported. Leafroller moths (Lepidoptera: Gracillariidae: Epicephala) are distributed throughout tropical and warm temperate regions of the Old World, where most are specialized pollinating seed predators (in a manner analogous to fig wasps or yucca moths) of their host leafroller plants (Phyllanthaceae: Phyllanthaeae). Mutualistic leafroller moths actively pollinate their hosts' flowers, but oviposit into the flowers' ovaries, where the larvae consume a subset of the hosts' seeds. This interaction is characterized by high biological intimacy, and thus has many parallels to fully symbiotic mutualisms. Multiple losses of pollination behavior—and therefore of mutualism—have been reported in the literature in leafroller moths in Asia.

Materials and Methods: Here we report the discovery of non-pollinating leafroller moths associated with native species of leafroller plants (*Nellica* spp., *Phyllanthus* spp., *Moeroris* spp.) in the southern United States (Oklahoma, Texas, Louisiana, and Florida). This is the first record of this high-intimacy plant-insect interaction from the Nearctic. Through phylogenetic inference of novel and previously published sequence data from this system globally, we infer the number of transitions to parasitism. We then

use these repeated transitions and phylogenetic comparative methods to ask questions about the changes associated with mutualism breakdown in the biology of leafroller moths.

Results: Phylogenetic analysis of data from the literature combined with novel sequence data from Asia and North America reveals that this mutualism has broken down into parasitism at least seven times, and possibly more frequently. This mutualism has thus evolved into parasitism more frequently than any other mutualism known to date. We find that in some of these lineages, loss of mutualism is associated with transitions among distantly related host plants and colonization of new continents.

Conclusions: These results indicate that some mutualisms are highly prone to breakdown, and that a macroevolutionary perspective on the fates of these parasitic lineages may reveal how mutualisms persist through evolutionary time. These results specifically suggest that mutualism breakdown may in some cases offer substantial expansion of ecological opportunity. However, as far as is known, mutualism loss only occurs from the insect side of the interaction; no losses of moth pollination are yet demonstrated in Phyllanthaeae. I then discuss the implications of these findings for studies of mutualism loss in other symbioses.

Reversal of host-parasite dynamics following an introduction of non-native species

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MEETING ROOM 113, August 18, 2025, 2:00 PM - 5:00 PM

Intimate interspecific relationships often exhibit coevolutionary dynamics, shaped by reciprocal adaptations and counter-adaptations. These interactions can shift along a continuum from mutualism to commensalism or parasitism. Biological invasions, by introducing novel partners in such relationships, provide valuable natural experiments to test predictions from ecological and evolutionary theory. We investigated how the invasion of the Chinese pond mussel (*Sinanodonta woodiana*), native to East Asia, affects the unique reciprocal relationship between the European bitterling (*Rhodeus amarus*) and native European unionid mussels. In this association, bitterling fish deposit their eggs in the gills of live mussels, while mussel larvae (glochidia) parasitize on fish fins and gills. In Europe, *R. amarus* is a parasite of mussels: it exploits all native mussel species for oviposition, while effectively resisting infestation by their glochidia. In contrast, *S.*

woodiana, which co-evolved with a diverse community of Asian bitterling species, exhibits strong defenses against such parasitism. Our experiments revealed that European *R. amarus* is unable to successfully use invasive *S. woodiana* as a host, as Asian *S. woodiana* consistently rejects the eggs of *R. amarus*. Conversely, *S. woodiana* glochidia successfully parasitize *R. amarus*, effectively reversing the traditional host-parasite roles observed in this system in Europe. These findings highlight how novel ecological associations produced by biological invasions can restructure established interspecific relationships and suggest potential consequences for the population dynamics of *R. amarus*. We then also experimentally explored and discuss implications of potential invasions by East Asian bitterling species on the nature of relationships and for the population dynamics of the European bitterling fish.

The Legacy of Past Heatwaves on 'Off-Host' Parasite Stages: Reduced Infection Risk and Costs in the Daphnia-Pasteuria system

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MEETING ROOM 113, August 18, 2025, 10:30 AM - 12:30 PM

Heatwaves challenge our understanding of how environmental stressors reshape host-parasite interactions, particularly during the critical "off-host" stages of endoparasites. These stages, often dismissed as inert and unaffected by changes in their environment, remain a blackbox in ecological research despite their key role in the life cycle of parasites. Here, we examined how heatwaves affect the infectivity and progression of infection of *Pasteuria ramosa*, a bacterial parasite of the planktonic crustacean *Daphnia magna*. In order to measure the respective influences of the bacterial genotype and the heatwaves, we exposed the off-host transmission stages (=spores) from four genetically distinct parasite isolates to three temperature regimes (20°C, 30°C, 40°C), and tested their subsequent effects on host and parasite traits. Heatwaves significantly reduced parasite infectivity, regardless of parasite genotype. Hosts infected with parasite

whose endured a heatwave, harbored abnormal early parasites stages (e.g., less cauliflowers and more grape formations), while late-stages of the parasite remained unaffected across all treatments, thereby ensuring stable reproductive output. Intriguingly, heat-stressed spores induced genotype-specific shifts in their host: one host clone increased castration rates at higher temperatures, while others experienced moderate to dramatic declines. Furthermore, individuals exposed to heat-stressed spores that remained uninfected, exhibited enhanced survival, suggesting reduced costs of resistance against damaged parasites. This study provides insights into how heatwaves, endured only by the parasite, can modulate various aspects of host-parasite interactions, underlying the importance of considering the parasite's environmental history in evolutionary ecology and epidemiology.

Tracing coevolutionary divergence in a host-parasite model system

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MEETING ROOM 113, August 18, 2025, 2:00 PM - 5:00 PM

A major driver of change in biotic interactions is the potential for coevolution between the interacting partners. Experimental coevolution studies have shown that coevolving populations often diverge from one another at the genetic scale, which might have knock-on effects on their future evolution. Identifying the cause of coevolutionary divergence is not trivial: is it caused by one partner, which then drives divergence in the other partner, or by both partners? We investigated this question in a host-parasite model system, the unicellular alga *Chlorella variabilis* and its lytic virus PBCV-1. Experimental coevolution in this system produces repeatable arms races at the phenotype scale (algal resistance and viral host range), but not at the genotype scale (algal and viral mutations). As viral characteristics typically promote parallel evolution, we hypothesized that the divergence of algae and viruses is driven by

an initial divergence in algal resistance functions. To test this, we separated and replicated the first steps of coevolution. We first evolved 22 strains of resistant algae, and showed that they belonged to at least three resistance types. Second, each resistant alga was used to produce a set of ~5 resistance-breaking virus isolates, which were sequenced and tested for parallel mutations. Surprisingly, viral mutations were not parallel: resistance-breaking viruses belonging to the same set could have mutations in genes affecting distinct virion structures (external glycans, hair-like fibers, binding site). Thus divergence in alga-virus coevolution could be caused by both partners. Future work will test the impact of this genetic and functional variation on the long-term evolution of the alga-virus interaction.



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S19 - Evolution of symbioses and interactions in microbial eukaryotes

A genomic approach to investigate the specificity of symbiont-conferred defence in the tripartite system aphid – *Hamiltonella* - parasitoid wasp

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POSTER SESSION 2 - **P02.113**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Parasitoid wasps exploit many insect species as hosts by injecting their eggs inside them, allowing the developing larvae to feed on and ultimately kill their hosts. Aphids, however, have evolved a unique strategy to counter parasitoid infections through a facultative symbiosis with the bacteria *Hamiltonella defensa*. The resistance against parasitoid wasps, provided by *Hamiltonella*, is hypothesized to be mediated by toxins, which arrest the development of wasp larvae and are encoded in the bacteriophage APSE associated with this endosymbiont. In *Aphis fabae*, *Hamiltonella*-conferred resistance to parasitoid wasps is highly specific: different combinations of *Hamiltonella* strains and parasitoid wasp genotypes result in higher or lower levels of protection to the aphid. Despite previous efforts, the genomic basis of *Hamiltonella*-mediated toxicity and its specificity in interactions with different parasitoids is not fully understood. To

unravel the genetic mechanisms involved in this tripartite system, we generated 14 high-quality genome assemblies of *Hamiltonella* strains isolated from diverse aphid populations and introduced them into a single clonal genotype of *A. fabae*. Phenotypic data and comparative genomic analyses of *Hamiltonella* and APSE aim to identify key genes and pathways involved in toxicity and in the resulting spectrum of protective phenotypes. Additionally, we examine the co-evolutionary dynamics between *Hamiltonella* and parasitoid wasps, investigating whether specific genomic features of *Hamiltonella* and APSE correlate with resistance to different parasitoid genotypes. This work sheds light on how genetic variation in *Hamiltonella* influences its defensive capabilities, advancing our understanding of the ecological and evolutionary pressures driving this intricate host-symbiont-parasitoid system.

Eco-Evo of Symbiosis: Environmental Heterogeneity Meets Density-Dependent Feedback

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POSTER SESSION 2 - P02.117, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Environmental heterogeneity, host movement, and density-dependent feedbacks are fundamental drivers of host-symbiont interactions, yet existing models rarely integrate all three concurrently. I introduce a spatially structured, two-habitat model that explicitly tracks uninfected hosts, hosts infected by either of two symbiont strains, and the corresponding pools of free-living symbionts in each habitat. This framework incorporates logistic growth for both hosts and symbionts, bidirectional migration between habitats, vertical transmission during host reproduction, and horizontal transmission via environmental exposure to the free-living pool.

In each habitat, hosts reproduce subject to a local carrying capacity that limits birth rates as population density approaches its maximum. A specified fraction of offspring inherit the symbiont from infected parents, ensuring vertical transmission, while the remainder join the pool of uninfected hosts. Uninfected hosts may acquire infection by encountering free-living symbionts shed continuously by infected individuals; these environmental transmission events follow a mass-action process. Infected hosts experience strain-specific mortality or fecundity adjustments, capturing potential costs or benefits of infection. Host mortality applies to all classes, providing turnover.

The symbiont component of the model consists of two free-living pools, one for each strain, in each habitat. Symbionts grow in the environment through logistic processes limited by a habitat-specific symbiont carrying capacity and decline due to natural decay. Infected hosts shed symbionts at a constant rate into the free pools, coupling host and environmental dynamics. Both hosts and symbionts migrate bidirectionally between the two habitats at defined rates, enabling spatial coupling, potential source-sink dynamics, and the study of spatial spread and persistence.

By coupling the four population classes across the two habitats through ordinary differential equations, the model yields a system that can be analyzed to derive invasion thresholds. And perform adaptive dynamics analysis to find evolutionary stable strategies for symbiont-host coevolution and symbiont transmission modes. .

These modeling and simulation components provide a flexible example for investigating how environmental heterogeneity and density-dependent processes shape the evolution of symbiotic interactions. I will present the model framework, analytical derivations of invasion metrics, and detailed plans for simulation experiments, highlighting opportunities for empirical validation and broader applications.

Few Targets but Broad Effects: Molecular Mechanisms of Host Phenotype Modulation by a Cestode Parasite

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POSTER SESSION 2 - P02.116, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The ability of parasites to modify their hosts' phenotypes has fascinated evolutionary biologists for a long time. Nevertheless, the processes – particularly the molecular mechanisms – that underpin these parasite-induced alterations remain frequently unresolved. The intricate nature of the interactions between endoparasites and their hosts raises the question of how they have evolved. Four different explanations for parasite-induced phenotypic changes in hosts have been put forward, these are: active manipulation, side-effects of manipulation, the involvement of third parties and the defence strategies of the host itself. A comprehensive understanding of these mechanisms is essential for elucidating how complex host phenotype changes are driven and maintained. We therefore set out to investigate these mechanisms in a system where the parasite (the cestode *Anomotaenia brevis*) has multiple profound phenotypic effects on its host (the ant *Temnothorax nylanderi*). Parasitic infection during the larval stage causes reduced melanisation and sclerotization of the ants' cuticle, increases its adult lifespan considerably, causes decreased activity and muscle dystrophia. These phenotypic changes go hand in hand with a large number of transcriptomic changes in the host. We thus set out to investigate the evolutionary strategies and molecular mechanisms underlying these alterations.

To investigate the mechanisms by which the parasite alters host phenotype, we conducted a series of experiments. We examined the parasite's impact on the host gut microbiome using amplicon sequencing, assessed the specificity of the host immune response through a full-factorial design combining cestode infection and a sham bacterial immune

challenge, and analyzed the resulting transcriptomic changes. Moreover, we developed a novel bioinformatic pipeline to link parasite gene expression to host gene expression, enabling the identification of cestode genes that influence specific host pathways.

We detected shifts in the gut microbiome of infected host workers, which showed reduced alpha bacterial diversity and were more frequently dominated by *Entomoplasma*. Whether these microbiome changes are directly caused by the parasite or indirectly by the observed increased longevity and care of infected workers remains unclear. However, they may contribute to behavioural modifications via the gut–brain axis. We also found evidence of immune suppression in infected hosts, with several immune pathways showing opposing expression patterns in response to cestode infection and bacterial challenge. Despite this suppression, antibacterial pathways remained inducible, indicating that the cestode allows the host to defend against microparasites. Integration of host and parasite transcriptomes using weighted gene co-expression network analysis revealed mechanisms of parasite-induced host modulation. Specifically, we identified statistical associations between parasite genes encoding secreted proteins and host gene expression, suggesting that the parasite targets oxidative stress responses, muscle activity, and the melanisation pathway—an important antiparasitic defence in insects.

Our findings indicate that complex phenotypic changes of hosts can be highly interconnected suggesting that parasites can induce extensive and coordinated changes of the host phenotype by targeting a limited set of molecular pathways.

Genetic variation among progeny shapes symbiosis in a basidiomycete with poplar

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POSTER SESSION 2 - P02.114, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Forest trees rely on ectomycorrhizal (ECM) fungi for acquiring scarce resources such as water and nutrients. However, the molecular mechanisms governing ECM traits remain inadequately understood, particularly the role of intraspecific fungal variation in root-tip colonisation and trophic interactions. This study examined six ECM traits using *Pisolithus microcarpus*, an ECM fungus capable of forming ECM rootlets in poplar. A collection of 40 sibling monokaryons and their parental dikaryon was analysed through genome and transcriptome sequencing to examine quantitative trait loci, gene expression, and mating-type loci. These findings revealed a pronounced phenotypic continuum in poplar root colonisation by sibling monokaryons, ranging from incompatible to fully compatible strains.

Genetic recombination among monokaryons was demonstrated, and genomic regions potentially involved in ECM-fungal traits were identified. Transcriptomic analysis revealed greater differentiation of transcriptomic profiles between fungal strains than between fungal tissues, and uncovered tissue-specific functional responses for ECM and free-living mycelia. Poplar exhibited distinct transcriptomic responses when interacting with different sibling monokaryons and the parental dikaryon. Allele sorting at 11 mating-type loci confirmed the species' heterothallic tetrapolar system. This study advances understanding of the genetic and transcriptomic mechanisms underlying ECM symbioses, highlighting intraspecific fungal diversity's role in forest ecosystem functioning.

Genomic signatures and evolutionary consequences of symbiosis in a beetle-associated fungus

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POSTER SESSION 2 - P02.111, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Insect-microbe symbioses enhance survival, expand ecological niches, and drive evolutionary change by enabling organisms to adapt to diverse environments. However, these relationships are inherently context-dependent, as exemplified by the association between the tortoise beetle *Chelymorpha alternans* (Coleoptera: Cassidinae) and the fungus *Fusarium oxysporum*. In this system, the fungus acts as a defensive ectosymbiont, protecting beetle pupae from predators. In return, the beetle propagates the fungus onto their shared host plant, *Ipomoea batatas*, where *F. oxysporum* switches to a phytopathogenic lifestyle. While the symbiosis is morphologically and functionally conserved across multiple tortoise beetle species, its origin and molecular evolution remained unclear. By integrating short- and long-read sequencing with phylogenetic inference, comparative genomics, and macrosynteny analysis, this study aims to reveal how these associations shape the genomic landscape

of *Fusarium*. Phylogenetic reconstruction of nine *Fusarium* symbionts from different beetle species demonstrate multiple independent acquisitions of the fungal symbiont by the host. The analysis supports an early, ancestral acquisition of the fungal symbionts across early-diverging tortoise beetles, followed by multiple independent replacement events in more derived lineages. Comparative genomic analyses further reveal drastically reduced genomes across these symbiotic species, including some of the smallest genomes assembled to date for a member of the *F. oxysporum* species complex. Lastly, macrosynteny analysis will guide our efforts to uncover large-scale genomic rearrangements and structural variations that are concomitant with a symbiotic transition. Together, these findings will offer key insights into the genomic evolution of *Fusarium*, which retains plant pathogenic potential in light of its association with the beetle.

Genomic signatures of adaptation to stress reveal shared evolutionary trends between *Tetrahymena utriculariae* and its algal endosymbiont, *Micractinium tetrahymenae*

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POSTER SESSION 2 - P02.110, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The evolution of intracellular endosymbiosis marks a major transition in the biology of the host and endosymbiont. Yet, how adaptation manifests in the genomes of the participants remains relatively understudied. To investigate this question, we sequenced the genomes of *Tetrahymena utriculariae*, a species-specific commensal of the aquatic carnivorous bladderwort *Utricularia reflexa*, and its intracellular algae, *Micractinium tetrahymenae*, and two of their closest relatives using long-read DNA sequencing. We discovered that the *T. utriculariae* genome experienced an expansion in a protein family bearing the TLD domain, which was also found to be under strong purifying selection, identifying it as a candidate for being an adaptive response to oxidative stress resulting from the physiology of its

endosymbionts. We discovered that the *M. tetrahymenae* genome is expanded relative to congeners and contains a greater number of rapidly expanding gene families. These were enriched for Gene Ontology terms relevant to cellular responses to stress and stimulus, and the regulation of intracellular signal transduction. Using a comparative transcriptomic analysis of *M. tetrahymenae*, we discovered that the algal genes that are differentially expressed between symbiotic and free-living states were enriched for pathways that evidence shifts in energy generation and storage and cellular protection. Together, our results elucidate the axes along which the participants must adapt in this young endosymbiosis and, in particular, identify adaptation to stress as a shared coevolutionary trend.

Host species identity and phyllosymbiosis as the main evolutionary and ecological drivers of gut microbiota in wild rodent species in the Yucatán peninsula

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POSTER SESSION 2 - **P02.115**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: The host-microbiome association is considered a coevolutionary process in which the microbiome provides important functions for host development, physiology, and health. However, the ecological and evolutionary forces shaping the diversity and structure of the bacterial communities that form the microbiome are still being elucidated. Microbiome studies in rodents suggest that the bacteriome is mainly shaped by diet, geography, host genetics, environment, and phyllosymbiosis. The latter refers to the eco-evolutionary pattern in which closely related host species often tend to have more similar microbiota. We assessed the composition of gut microbiota in six rodent species from three geographic regions across the Yucatán peninsula, Mexico. We aimed to determine if the host species identity and phyllosymbiosis are the primary drivers of gut microbial structure and diversity. We predict that each host species would have an overall distinct bacterial community sharing the same environment.

Materials and methods: We amplified and sequenced the 16S rRNA V4 gene in 63 rodent individuals and evaluated the contribution of host species identity, phylogenetic relationships, habits, and geography to the rodents' gut microbiome. For this, we quantified Hill numbers for alpha diversity and assessed differences in abundance of microbial taxa among host species. We used a random forest approach to analyze, identify, and classify the bacterial families between rodent species based on their similarity. We also estimated beta diversity with phylogenetic and non-phylogenetic estimators and evaluated differences among host species. We applied Blomberg's K and Pagel's λ tests to determine whether microbial diversity, phylogeny and beta dispersion have a phylogenetic signal. Finally, we performed a Mantel test between host phylogenetic distances and bacterial community dissimilarity.

Results: Significant differences ($p < 0.01$) among rodent species were found for amplified sequences

variants (ASVs), where *Ototylomys phyllotis* and *Sigmodon toltecus* showed the highest richness and *Peromyscus leucopus* the lowest across all taxonomic levels. No differences were observed for common and dominant microbial taxa for any taxonomic category. The host species had differential abundance, where Lactobacillaceae was highly abundant in *Peromyscus yucatanicus*, Christensenellaceae predominating in *P. leucopus*, Muribaculaceae in *O. phyllotis*, Ruminococcaceae in *Heteromys gaumeri*, Desulfovibrionaceae in *Oryzomys couesi*, and Staphylococcaceae in *S. toltecus*. Random forest (RF) classifier was able to categorize the individuals into their respective species with high accuracy (94%), indicating that host species determine microbial composition. Phylogenetic distances (Unifrac) showed significant composition differences between *P. yucatanicus* and *O. phyllotis* and between *P. yucatanicus* and *S. toltecus*. Finally, we found weak but significant phylogenetic signals on microbial richness, common species, beta dispersion, and beta diversity.

Conclusions: Our results show that host species identity and phyllosymbiosis are the main mechanisms driving microbiome dissimilarity across the rodent species studied. Specifically, that gut bacterial assembly dissimilarity increases with host phylogenetic distance, which could imply reciprocal adaptation between microbiota and host. Notably, host phylogeny constrains interspecific composition but not abundance; we found that the main gut microbiota differences between rodent species were based on the abundance of eight bacterial families, which are associated with distinct habitats, diets, and metabolic functions. For instance, rodents with more specialized habits like *Ototylomys phyllotis* (semi-arboreal and folivorous) have higher bacterial diversity. The multispecies approach allows us to make inferences about their evolution and ecology.

Intracellular signaling in proto-eukaryotes evolves to alleviate regulatory conflicts of endosymbiosis

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*POSTER SESSION 2 - **P02.112**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM*

The complex eukaryotic cell resulted from a merger between simpler prokaryotic cells, yet the role of the mitochondrial endosymbiosis with respect to other eukaryotic innovations has remained under dispute. To investigate how the regulatory challenges associated with the endosymbiotic state impacted genome and network evolution during eukaryogenesis, we study a constructive computational model where two simple cells are forced into an obligate endosymbiosis. Across multiple in silico evolutionary replicates, we observe the emergence of different mechanisms for the coordination of host and symbiont cell

cycles, stabilizing the endosymbiotic relationship. In most cases, coordination is implicit, without signaling between host and symbiont. Signaling only evolves when there is leakage of regulatory products between host and symbiont. In the fittest evolutionary replicate, the host has taken full control of the symbiont cell cycle through signaling, mimicking the regulatory dominance of the nucleus over the mitochondrion that evolved during eukaryogenesis. Thus, our modeling approach contributes to an integrated view of the eco-evolutionary dynamics of endosymbiosis and the transition towards individuality of the symbiotic partnership.

An evolutionary mosaic of proteins mediates metabolic integration in transient kleptoplasts in the euglenozoan *Rapaza viridis*

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MEETING ROOM 131, August 19, 2025, 11:00 AM - 1:00 PM

Acquired phototrophy is widespread among various eukaryotic lineages and involves different secondary endosymbioses of photosynthetic, plastid-bearing eukaryotes. While most of these secondary photosymbioses preserve the wholeness of the photosynthetic endosymbiont, others only retain determined endosymbiont organelles. To the extreme of this range, in kleptoplastidy, the host subtracts solely the plastids from the endosymbiont, which remain only temporarily functional and thus require constant replacement. While kleptoplastidy is broadly present among eukaryotes, we only know about four examples in which the host, at least partially, controls kleptoplast activity with nucleus-encoded proteins. The euglenozoan *Rapaza viridis* is a unique model of obligatory kleptoplastidy, showing high specificity for the plastids of one particular green alga, *Tetraselmis* sp. NIES-4478, and thriving only in the presence of both light and prey algae. Furthermore, transcriptomic data revealed extensive targeting of nucleus-encoded proteins to the kleptoplast, suggesting an extraordinary molecular integration. Here, we study the molecular integration of the kleptoplasts in *Rapaza viridis* metabolism by predicting the plastid proteome from the *Rapaza viridis* transcriptome and reconstructing plastid pathways. We combine this information with the plastid proteomes of eight photosynthetic euglenozoans with permanent plastids predicted from newly generated and publicly available transcriptomes. We further study the evolutionary origin of over 200 detected proteins involved in seven putative plastid metabolic pathways, the photosynthetic machinery, and transporter elements.

The nucleus of *Rapaza viridis* encodes kleptoplast-targeted proteins involved in functions related not only to photosynthesis but also to other processes occurring in plastids,

such as carbon fixation or chlorophyll, terpenoid, and carotenoid biosynthesis. However, the completeness of these pathways is uneven, and some essential proteins are missing in the *Rapaza viridis* proteome. Phylogenetic analyses revealed further insights into the origin of these proteins, describing three different evolutionary scenarios. First, some proteins showed an unambiguous origin in the species from which *Rapaza viridis* steals the plastid, most probably acquired by endosymbiotic gene transfer. Variable divergence levels (comparing *Tetraselmis* sp. and *Rapaza viridis* sequences) revealed that the acquisition of these genes occurred at different times. The second scenario encompasses the proteins acquired from species other than *Tetraselmis* sp., either by endosymbiotic or horizontal gene transfer. In euglenozoans, nucleus-encoded proteins require a spliced leader trans-splicing pre-sequence, essential in the posterior RNA regulatory process. Remarkably, genes coding proteins in these two evolutionary scenarios showed the development of this spliced leader sequence, confirming the complete integration in the *Rapaza viridis* nucleus. The third scenario comprised a set of *Rapaza viridis* own proteins that seem to have been re-targeted to the kleptoplast in the switch to mixotrophy.

Kleptoplastidy is an advantageous model for studying mechanisms of plastid integration in eukaryotes. However, most known examples involving ancestrally-lacking-plastids species do not usually detect significant targeting of nucleus-encoded proteins, suggesting limited molecular integration. Comparatively, *Rapaza viridis* kleptoplasts show substantial metabolic integration, standing out as a new model to investigate the early and intermediate stages of plastid integration and opening a new gate to experimental research in this field.

Interplay of HGT and targeting during the chromatophore organellogenesis in Paulinella

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MEETING ROOM 131, August 19, 2025, 11:00 AM - 1:00 PM

Horizontal Gene Transfer (HGT) is the movement of genes between genomes apart from sexual recombination. Eukaryotic and prokaryotic co-occurrence, symbiotic relationships or phagotrophic lifestyles can modulate HGT. The chromatophore is a photosynthetic organelle in some Paulinella species, it derives from an endosymbiotic event that occurred more recently than the origin of chloroplasts. Thus, Paulinella represents a crucial clade to understand organellogenesis. HGT events have been previously described between Paulinella and diverse bacteria. However, an in-depth characterisation of the donors and a temporal framework for transfer highways in

these species is still missing. Here, we studied the origin of the gene families in the species Paulinella micropora, assessed HGT patterns, and inferred the relative age of the transfers from non-eukaryotic clades to evaluate whether HGT happened in distinguishable waves. We observed a high diversity of donors, which transferred genes in a continuum rather than specific waves. Functional and subcellular localization analyses show that the majority of the chromatophore-targeted proteins have a vertical origin. Based on this and other results, we propose a model for the origin and early evolution of the chromatophore.

Stepwise genome evolution from a facultative symbiont to an endosymbiont in the N₂-fixing diatom-Richelia symbioses

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MEETING ROOM 131, August 19, 2025, 11:00 AM - 1:00 PM

Cyanobacteria from genera *Richelia* are predominantly observed in symbiosis with diatoms, particularly in nitrogen-poor regions of the ocean. The diatom-*Richelia* symbioses represent a valuable system to study the evolution of symbiosis from free-living and facultative cyanobacteria to obligate symbionts. A unique feature of this system is the continuum of symbiont cellular integrations ranging from externally attached epibionts to periplasmic symbionts and endobionts, accompanied by corresponding patterns of genome reduction and functional gene loss. In this study, we analyzed four draft *Richelia* spp. genomes and ten environmental metagenome-assembled genomes (MAGs) using comparative genomics analyses. We observed an enrichment of insertion sequences (IS) and high numbers of pseudogenes in the genomes of periplasmic symbionts, suggesting earlier stages of genome reduction. Conversely, endobionts exhibited comparatively smaller genomes, fewer IS

and pseudogenes, consistent with advanced stages of genome degradation and increased host dependency. Pangenome analyses revealed that endobionts maintain a higher proportion of their gene clusters in the core genome, while epibionts and periplasmic symbionts possess more flexible and singleton gene clusters, indicating greater genomic plasticity. Functional comparisons with other N₂-fixing cyanobacteria, including the spheroid bodies of diatom *Epithemia*, UCYN-A/nitroplast, and *Nostoc* azollae symbiont of water ferns, showed similar patterns of metabolic gene loss. However, *Richelia* endobionts were distinguished by the specific absence of genes such as cytochrome bd ubiquinol oxidase and those required for the biosynthesis of lipid A, α -Tocopherol, biotin, and tryptophan. These losses highlight increased host dependency and a trend toward irreversible genomic reduction.

Subcellular proteomics illuminates the relationship between host and symbiont in trypanosomatid *Angomonas deanei*

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MEETING ROOM 131, August 19, 2025, 11:00 AM - 1:00 PM

Kinetoplastid *Angomonas deanei* harbours a single endosymbiont of β -proteobacterial origin within its cytoplasm. This endosymbiont provides various metabolites such as heme and purines, and occupies a consistent position within the cell, undergoing coordinated replication prior to that of its host. *A. deanei* encodes seven known endosymbiont-targeted proteins (ETP), which localise to various regions of the bacterium and exert control over endosymbiont-related processes, including division. Gene loss undergone by this bacterium renders it unable to survive as a free-living organism, and it is regarded as being in a transitional state from endosymbiont to integrated organelle, like the mitochondrion or plastid.

To better clarify the relationship between both organisms, we initiated subcellular proteomics to assign localisation for the proteins encoded by each, many of which

lack any sort of functional annotation. Through our efforts, we confirm the existence of an eighth ETP and identify several other candidate proteins being targeted from the host to endosymbiont in a similar manner. We further identify a novel lateral gene transfer to the nuclear genome, encoding a protein interacting with a previously identified ETP, which regulates endosymbiont division. Finally, we proteomically characterise a 'contact site' between the bacterium and host organelles, primarily consisting nuclear membrane and endoplasmic reticulum constituents, which seemingly serves to maintain the endosymbiont in its characteristic position. Our data suggests the employment of over a hundred proteins by *A. deanei* to maintain this complex relationship and offers insight into the nature of endosymbiotic events seen across protistan organisms.

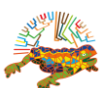


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S20 - Evolutionary biology meets genetic pest control



Epistasis among fungicide resistance mutations

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POSTER SESSION 1 - P01.238, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Fungicide resistance poses a significant threat to agricultural productivity, as fungal pathogens increasingly adapt to withstand fungicides. Understanding the genetic interactions, or epistasis, among fungicide-resistant mutations is crucial for predicting the

evolutionary trajectories of resistant strains and informing sustainable control strategies. In this study, we investigated the epistatic interactions among resistance mutations by evolving 20 lines of the filamentous fungus *Neurospora crassa*, with 10 lines for each mating type, under incrementally increasing concentrations of the fungicide tebuconazole, culminating at 2 µg/ml. Following the evolution experiment, we will sequence the

genomes of the evolved lines to identify mutations conferring resistance. Crosses will be then made between lines harboring distinct resistance mutations to evaluate the extent to

which epistasis influences the fitness of resistant strains. By quantifying growth rate variations among offspring with different combinations of resistance mutations, this research aims to clarify whether epistasis hinders, enhances, or has a neutral effect on fungal growth rate in the context of fungicide resistance. These findings will contribute to our understanding of resistance dynamics and provide insights into the potential limitations and strengths of resistant fungal populations under ongoing selective pressures.



Implications of intraspecific variation in genes determining host plant use in a generalist herbivore

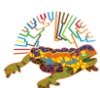
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POSTER SESSION 3 - **P03.033**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The interactions between plants and herbivores fuel an evolutionary arms race, evidenced through ecological and molecular scales. Anti-herbivory strategies of plants impose pressure on herbivores to evolve counter adaptations. In agricultural ecosystems, pest control strategies, such as chemical control and breeding of resistant plant varieties, are constantly compromised by pest adaptations due to natural selection. Knowledge about the adaptability of pests can help prevent pest formation, before the need to control pest outbreaks arises. We investigate the molecular interactions between the generalist mite *Tetranychus urticae*, a notorious agricultural pest, and several of its plant hosts. During feeding, mites inject salivary proteins into their hosts. These proteins interact with targets in the plant, subsequently inducing or suppressing plant defenses against herbivory. Our aim is to explore how intraspecific variation in mite genes coding for salivary proteins affects their ability to interact with plant targets, in order to estimate how these protein interactions promote adaptation to novel hosts. We conduct a comprehensive field

effort to sample mite individuals from different host plant species across the dune ecosystem of the Netherlands and Belgium, in order to identify natural variants of a spider-mite specific family of genes encoding for salivary proteins, called “SHOT”. We isolate DNA from individual mites and obtain long-read sequences of the SHOT2 clade, which consists of four paralog genes residing in a 25kb region of the spider mite genome. Using an array of protein-protein interaction techniques, we identify a target of a SHOT2 protein and established that in absence of this plant target, mite fitness decreases. We assess to which extent different SHOT variants (de)stabilize the interaction with different target orthologs, and how this relates to mite fitness using SHOT2-mutant mite lines. We provide empirical evidence of the relationship between allelic variation in herbivore genes and plant targets that determine host compatibility, which is crucial for data-driven crop management strategies.



Insect-plant interactions at cell-type resolution: insights from the green-veined white

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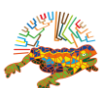
POSTER SESSION 1 - **P01.239**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Phytophagous insects such as the butterflies have evolved detoxification genes and gene families in response to the chemical defenses of their host-plants. In particular, pierinae butterflies (Lepidoptera: Pieridae) are well known to resist the glucosinolate (GSL) defenses of their host-plants belonging to the cabbage family (Brassicales) and are optimal research organisms for plant-insect interaction studies. Although transcriptomics has allowed the discovery and characterization of several gene families involved in detoxification, little is known about their spatio-temporal expression across cell lineages. Here we applied single-nuclei RNA sequencing (snRNA-seq) to characterize the cell types present in the midgut of *Pieris napi* larvae that were fed two Brassicales host plants. Our goal was to search for differentially expressed genes due to the diets and to assess their expression patterns across identified cell lineages/types. **Materials and Methods:** *Pieris napi* larvae were reared on either horseradish (*Armoracia rusticana*) or watercress (*Nasturtium officinale*) leaves. At 4th instar, midguts were extracted and flash frozen to be processed for snRNA-seq following 10x Genomics technologies and protocols. Short sequencing reads were mapped to the *P. napi* reference genome and the resulting gene expression matrix was filtered and processed for downstream analysis using the bioinformatics tool Seurat, which clusters the cells according to their transcriptomic profiles.

Results: From our cell transcriptomics analysis we identified intestinal stem cells as well as the lineages leading to enterocytes and enteroendocrine cells. In addition, we identified cells from the immune system and the midgut muscle and neuromuscle. We discussed the reliability of the proposed

midgut cell type markers from the fruit fly *Drosophila melanogaster*, the silk moth *Bombyx mori* and the fall armyworm *Spodoptera frugiperda*, and then assessed their applicability to pierinae butterfly mid-late stage larvae, and described a series of candidate marker genes found in our dataset. Regarding differential gene expression caused by diet treatment, we found that major allergen (MA), a gene coding for nitrile-specifier proteins (NSPs) known to confer resistance to GSL, was highly upregulated across all cell clusters in watercress-fed samples (WC), predominantly in those identified as enteroblasts. However, this gene is strikingly downregulated in all clusters from the horseradish treatment (HR). This diet-derived differential expression pattern is also observed for several cytochrome P450-like coding genes, known for their role in insecticide metabolism, and other known detoxification gene families, namely, UDP-glycosyltransferases (UGTs), sulfotransferases, carboxylesterases and glutathione S-transferases (GSTs). Interestingly, some GST-like genes show an opposite expression pattern (high in HR, low in WC). While most of these detoxification genes are significantly upregulated in WC compared to HR and their activity appears to be general regardless of cell lineage/type, some genes show cell type specific expression in one or the other treatment. This is also observed for certain genes with no known role in detoxification.

Conclusion: Our findings provide a plethora of candidate genes for midgut cell-type identification as well as cell resolution insights into the expression and role of detoxification gene families showing the benefits of applying snRNA-seq in insect-plant interaction research.



Integration of databases and High-Performance Computing in Dengue Virus research: Advances in the development for RT-LAMP detection

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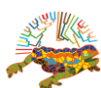
POSTER SESSION 1 - P01.235, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Introduction: Dengue virus (Orthoflavivirus denguei) is a single-stranded RNA-positive flavivirus (~11 kb) with four antigenically distinct serotypes. Its transmission by mosquitoes, mainly *Aedes aegypti* and *A. albopictus*, explains the 14 million global cases and 7,700 deaths reported in 2024. In Colombia, an increase of patients with Dengue symptoms was observed in 2023 and, for the department of Santander, in 2024. Therefore, the National Institute of Health of Colombia invited to improve the methods of its diagnosis, for which it is critical to optimize molecular diagnostic methodologies such as ELISA, RT-PCR and RT-qPCR, that will always be limited by gene drift and selective pressures on the genomic regions used for virus identification. Another option is RT-LAMP, that has the advantage of simplicity and speed due to its thermo-dependence and colorimetric readout. However, it also requires primers designed under rigorous evolutionary parameters to minimize false negatives. This study proposes an integrative bioinformatics protocol for RT-PCR/RT-LAMP oligonucleotide design based on evolutionary patterns of the virus in South America. **Material and Methods:** Complete Dengue genomes were collected from the GenBank database up to December 2024. With an R algorithm optimized for High-Performance Computing (HPC), sequences with ambiguous regions (>5% N), low quality (Phred <30) and indeterminate regions (-) were filtered out. The final database included sequences from South America. The alignments were performed in MAFFT with the parameters of the evolutionary model of DNA loss. Consensus sequences were generated with BioEdit: (1) majority consensus (nucleotide frequency >70%); and (2) degenerate consensus (IUPAC code for polymorphisms with frequency >10%). RT-PCR primers were designed with the OligoAnalyzer tool, while RT-LAMP primers were designed with the NEB LAMP Primer Design Tool

(parameters: Tm=65±2 °C, length 18-22 nt, GC=30-70%), and the in silico specificity was validated through BLASTn.

Results: 7,490 complete Dengue genomes were obtained, the alignment by serotype showed a world regional distribution. Therefore, to determine the previous pattern, 219 sequences were selected that met the selection criteria: Colombia, Ecuador, Panama, Venezuela, Peru and Brazil. The consensus sequence generated by IUPAC showed a high nucleotide variability. However, the majority nucleotide consensus (>70% frequency) identified 11 conserved genomic regions within the ORF. These regions were used to design 8 RT-LAMP primer sets, 2 per serotype, DENV-1 to DENV-4. In silico validation by BLASTn showed that the primers presented differential coverage between serotypes (> 95%), with no cross-reactivity with human genomes or other pathogens. Additionally, primers for real-time RT-PCR that target conserved segments were proposed and corroborate the quality regions.

Conclusion: The integration of HPC applied to RT-LAMP primer design for the identification of conserved genomic regions of dengue virus serotypes allows for the design of oligonucleotides and hybridization parameters for viral genetic RT-LAMP, which will reduce false-negative results. Furthermore, complementing the results with RT-PCR and the characterization of new viral genotypes with Next-Generation Sequencing will contribute to global and regional epidemiological surveillance by identifying emerging viral variants. The authors are grateful to the Internal Call for Research, with Cash Contribution of the E.S.E. Hospital Universitario de Santander 2024. Mobility Program and Project No. 8078 of Vicerrectoría de Investigación y Extensión of the Universidad Industrial de Santander, for funding this research.



Limited spatial propagation of quorum-drive systems through wave pinning

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POSTER SESSION 1 - **P01.242**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Artificial gene drive alleles are engineered to bias their own inheritance, and can help rapidly spread a desired trait through a target population. Spatial confinement is essential to restrict gene drive spread to target populations and prevent unintended ecological impacts. It can be obtained via the use of a bistable system, in which an allele can only spread if introduced above a threshold frequency, and if the migration rate is low enough. Bistability is produced by a fitness valley, which can be caused by heterozygote disadvantage or the use of toxin-antidote systems. In a metapopulation, the spatial spread of a bistable drive allele can be limited. This phenomenon is called "wave-pinning" in traveling wave models for spatial invasion. However, predicting when waves will stop is difficult. Here, we first

adapt existing methods for wave pinning prediction in bistable one-locus heterozygote systems. We then extend the study to a two-locus four-allele drive system. We show that wave pinning appears as a result of limited dispersal. We also find that wave pinning can artefactually happen as a consequence of changing the precision of the discretization lattice in the numerical simulation of partial differential equations, and we provide an upper bound on lattice size to prevent this numerical artifact. Relying on incorrect numerical models to inform gene drive policies could have far-reaching consequences on natural populations; our results describe a way to avoid incorrect predictions of spatial confinement of introduced gene drive alleles



Optimizing biological pest management by investigating the genetic basis of parasitoid chemical communication systems

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POSTER SESSION 1 - **P01.244**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

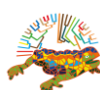
Parasitoid wasps are increasingly recognized as effective biological control agents in integrated pest management, offering sustainable alternatives to chemical pesticides with benefits for human health, agricultural productivity, and ecosystem integrity. A detailed understanding of their pheromonal communication—central to reproduction and species interactions—is essential for maximizing their efficacy as biological control agents.

The *Nasonia* species complex, a powerful model system in both evolutionary and chemical ecology, offers key insights into how complex mating signals evolve and function. In *Nasonia*, females produce species-specific cuticular hydrocarbon (CHC) profiles that serve as sex pheromones, guiding male courtship and mating behavior. We identified specific methyl-branching patterns in these CHCs as critical components of sexual signaling and functionally characterized three fatty acid synthase genes responsible for their biosynthesis. These genes maintain both overall CHC profile integrity above male detection thresholds and the specific structural features linked to sexual

attractiveness—directly connecting genetic variation to mating success.

Our findings demonstrate how genetic information can be translated into ecologically relevant chemical signals, revealing a relatively simple genetic basis for sexual attractiveness. They also provide rare insights into the potential evolution of sexual communication and its role in reproductive isolation and speciation. Building on this foundation, we are now investigating the chemosensory mechanisms underlying pheromone perception in the *Nasonia* model system, which is exceptionally well-suited to uncover these understudied processes as well to obtain a more holistic view of the delicate interplay between sender and receiver.

By elucidating the genetic and sensory basis of pheromone communication, our research advances both fundamental evolutionary biology and the applied science of biological pest control. This integrative approach holds promise for optimizing mass-rearing conditions and sustainability over multiple generations of parasitoids like *Nasonia*, contributing to more efficient, eco-friendly strategies for sustainable agriculture.



Polymorphism Of The Ovine-Mhc Drb1 Exon 2 Gene And Its Association With Tick Infestation In Damara Sheep At Neudamm Farm, Namibia

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POSTER SESSION 1 - **P01.237**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The objective of this study was to identify allelic variation in the ovine-MHC DRB1 exon 2 gene and investigate the relationship of the polymorphism to external tick infestation in Damara sheep at Neudamm farm. The study also investigated sex and season effects variation in tick numbers. Ninety sheep (n=64 female and 26 male) were used. The ticks were counted twice in two seasons, wet and dry. Blood samples were collected in four mL K2E-EDTA anti-coagulant vacutainer tubes via jugular vein. Genomic deoxyribonucleic acid (gDNA) was extracted from blood using ZR Zymo kit (Zymo Research, CA, USA). Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method was used to identify genetic variation in the MHC DRB1 exon 2 gene. Chi-square test was used to test for association at 5% level of significance and to determine

the significance ($P < 0.05$) of mean variations of tick numbers between seasons. Three banding patterns, representing three genotypes, A1A1 (30%), A1A2 (40%), A2A2 (30%) were observed. A1A1 genotype had high tick counts than the other genotypes. However, this was not statistically significant. Similarly, there was no significant difference between the male and female tick counts ($P > 0.05$). Tick numbers differed significantly between the wet and dry seasons ($P < 0.05$). The genotypes and allele frequencies of the gene and number of ticks were in Hardy-Weinberg equilibrium (HWE). These preliminary results may be used for future genetic and conservation research to identify genetic markers linked to tick infestation in Damara sheep.

Key words: Damara sheep, PCR-RFLP, Polymorphism, Restriction enzyme, BsaHI.



Spatial soft-sweeps: a means to jointly estimate recent demography and migration in *Anopheles gambiae* from insecticide resistance loci

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POSTER SESSION 1 - **P01.243**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Predicting the response of populations to genetic interventions, and ecological changes in general, require a characterisation of their population size and migration rates, as these determine the complex spatio-temporal dynamics of populations — for example, chasing dynamics seen in response to suppression gene drives (North et al, BMC Biol, 2019, 2020 and Champer et al, Mol Ecol, 2021) — and ultimately the probability and duration of resistance (Khatri & Burt, PNAS, 2022).

Spatial soft sweeps give an opportunity to probe the recent evolutionary history of natural populations. We recently developed a robust method to estimate the recent effective population size from the number of independent origins of a positively selected mutation (Khatri & Burt 2019, MBE), assuming a panmictic population. However, in practice, local migration will give rise to a complicated spatial structure of origins, which may leave a fingerprint for its inference.

Here we develop computationally efficient simulations of spatial soft sweeps, which go

beyond the assumption of allelic exclusion (Ralph & Coop, Genetics, 2010; Paulose 2019). By a judicious choice of summary statistics, we show that these simulations can jointly estimate population size and diffusion constant, using a combination of ABC and machine learning approaches.

We apply this method to the *kdr* insecticide resistance mutation in the *Vgsc* locus in natural populations of *Anopheles gambiae*, across the whole range of sub-Saharan Africa, to give for the first time a continent wide picture of their recent evolution. Our results suggest effective population sizes of order 100s millions and diffusion constants of order 100s of km²/generation. Future work will focus on refining these estimates in light of seasonal variation in demography and the role of non-local migration.

These results will inform future stochastic spatio-temporal modelling of suppression drives to more accurately predict the response of natural *Anopheles* populations to these genetic interventions.



Sting Operation: Population Genetics of Yellow Legged Hornets (*Vespa velutina*) Using Single Nucleotide Polymorphisms

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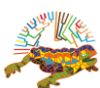
POSTER SESSION 1 - **P01.236**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Yellow legged hornets (*Vespa velutina*) are invasive insects which pose a threat to native insects, biodiversity and apiculture. They were thought to have first been introduced to France in 2004 in a shipment of pottery from Asia. In the following years, these insects have rapidly spread across Europe to countries such as the UK, Spain, Portugal, Italy, the Benelux countries, and Germany.

In the UK, there have been 147 laboratory confirmed sightings since 2016, including 110 nests. The government is currently taking an eradication approach, where each identified nest and individual are destroyed. It is thought that the majority of hornets intercepted in the UK are hitchhikers from the continent but there is suspected local breeding taking place as well. The UK government also makes use of genetic markers (microsatellites) to inform management. These markers are used to assign any lone hornets caught in traps away from the nest to any incepted nests already located. If the individual cannot be assigned it can indicate the presence of a new nest within that area suggesting the need for increased monitoring and trapping. The microsatellites markers are also used to

determine the number of males have bred with the queen to produce the nest.

Microsatellites however have limited resolution for kinship analysis and make use of older sequencing technologies. Therefore, a more precise method involving the use of high-density genetic markers, such as single nucleotide polymorphisms (SNPs), and next generation sequencing techniques (Illumina sequencing), could provide a higher resolution solution. Single nucleotide polymorphisms are variations at single base position in the DNA. Hundreds of SNPs can be amplified within a Genotyping in Thousands by Sequencing (GT-seq) assay (Campbell et al. 2015) to have the potential to genotype multiple individuals simultaneously at lower costs and with higher resolution. This study developed a panel of hundreds of SNP markers which were sequenced using Illumina MiSeq to analyse UK nests and individuals from 2016 - 2024 and comparing their genetics to European samples (Jersey, France, Benelux, Germany, and Portugal), improving our understanding of hornet spread and kinship relationships between nests aiding in management strategies within the UK and beyond.



Thermal adaptation amplifies increases in insect crop damage from climate warming

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POSTER SESSION 1 - **P01.246**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

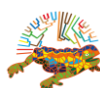
Climate warming can influence insect populations through direct effects on physiological processes, like those governing development, growth, and reproduction. Such plastic responses are projected to increase crop damages by insects under future warming scenarios. However, previous work has largely overlooked the important role of adaptive evolutionary responses of insect pests facing climate change.

Here, we evolved replicate lines of the seed beetle pest *Callosobruchus maculatus* under 23 °C, 29 °C (“ancestral”), and 35 °C to assess evolutionary potential in temperature-dependent reproductive output, development rate, and growth rate. To estimate future population growth and crop damage rates across seasons, we integrate evolved reaction norms with diverse realistic and temporally high-resolved thermal regime simulations under different warming scenarios. In line with previous research, we find that plastic responses on average increased crop damage by approximately 20% per degree of warming. Crucially, however, we find that projected crop damage is on average an additional 64% higher in the warm-adapted selection lines than in the ancestral lines. This increase from only 10 years of thermal adaptation in the laboratory corresponds to that caused by a mean climate warming of ~3

°C. Additionally, by fixing reaction norms for certain traits to the ancestral state and allowing others to take on the evolved state, we identify counter-gradient shifts in maximum growth rate and reproductive output as the main culprits behind the increased crop damage rates in the warm-adapted lines.

Moreover, we show that diurnal and seasonal temperature variability are crucial for understanding the dynamics of future insect populations due to the nonlinear and asymmetric nature of thermal reaction norms. For example, already warm sites may become inhospitable in summer due to daytime extremes exceeding thermal limits. However, these losses may be offset by earlier springs and prolonged falls, extending the growth season. Studies fixing the growth season to a certain period of the year might therefore underestimate crop damage increases in warm climates.

In summary, we show that evolutionary responses to high temperatures are likely to amplify climate-driven increases in crop damage beyond previous estimates. Our results highlight the need for pest managers and risk analysts to account for evolutionary change and natural thermal variability at relevant regional scales for effective forecasting and control of future pest populations



Transcriptional profiles of the fish parasite *Neoechinorhynchus agilis* (Acanthocephala) emphasize energetic stress in males and high cell-division activity in females

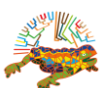
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POSTER SESSION 1 - **P01.240**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Acanthocephalans are parasitic worms with a complex life cycle consisting of a larval phase in a mandibulate arthropod and a reproductive phase in a gnathostome vertebrate. They present peculiar features, such as the ability to alter their intermediate host's behavior, thereby increasing their transmission probability, and the ability to accumulate heavy metals from the aquatic environment, lowering their concentration in fish hosts. In addition, these helminths can be pests in South American aquacultures. Although they make excellent models for the study of host manipulation and bioaccumulation, as well as of the evolution of parasitic lifestyle (e.g. loss of digestive tract), molecular data is scarce, and, to date, only one nuclear genome has been published. Overall, transcriptional bases of most aspects of the acanthocephalan life cycle are vastly unknown. Our contribution to filling this gap involves the de novo assembly of a nuclear draft genome and of the transcriptome of *Neoechinorhynchus agilis* (Eoacanthocephala) worms extracted from thinlip mullets' intestines. We conducted

differential expression analyses to compare male and female transcriptional profiles, as well as of worms of different sizes, to gain insight into the gene expressional background of maturation and reproduction in these parasites. Overall, male-biased transcripts were annotated with ontologies associated with energy metabolism, cell motility, and microtubules, suggesting male competition and sperm production. Genes related to response to stimulus and to stress were also male-biased, especially in the smallest worms, potentially illustrating the arrival of young individuals searching for a favorable position in the intestines. Gene ontologies referring to transcription, cellular division, development and adhesion, and DNA damage response were over-represented in female-biased genes, congruently with massive egg production and embryo development. Finally, we highlight fifteen genes the expression of which shows a linear correlation to size in both males and females and have human homologs involved in cancer, evidencing their importance in development and their relevance as potential targets for pest control.



Assessing the role of mitonuclear and genotype-by-environment interactions for the Trojan Female Technique in pest biocontrol

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MEETING ROOM 118+119, August 18, 2025, 10:30 AM - 12:30 PM

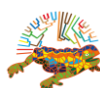
The seed beetle (*Acanthoscelides obtectus*) is a globally distributed pest affecting common beans and various legume species. It feeds on seeds and causes considerable damage to stored products, resulting in substantial economic losses. Currently, insecticides are mainly used to control this species. However, the detrimental effects of these chemicals on the environment and non-target species, as well as the evolution of resistance, necessitate the development of safer and more specific biocontrol methods.

One such method is the recently proposed Trojan Female Technique (TFT), which leverages the evolutionary phenomenon known as the Mother's Curse. This phenomenon predicts the accumulation of male-only harming mutations in the mitochondrial genome due to its matrilineal inheritance. The release of Trojan Females - females carrying naturally occurring mitochondrial genotypes (mitotypes) that negatively impact male reproductive potential - facilitates self-sustaining transgenerational population suppression. A candidate TFT mitotype that induces male

subfertility has recently been identified in seed beetles.

However, a crucial criterion for the successful application of TFT is that the TFT mitotype must render males subfertile across a range of nuclear and environmental backgrounds, such as different temperatures. To investigate this, we tested the effects of the candidate TFT mitotype (MG3b) and two control mitotypes (MG1a and MG1d) on male and female fertility in three different nuclear backgrounds and under two temperature regimes.

Our results show that the candidate TFT mitotype at standard temperature reduces male fertility across all nuclear environments compared to control mitotypes. Conversely, MG3b males have similar reproductive performance to MG1a and MG1d males at elevated temperatures. On the other hand, female beetles carrying MG3b remain fully fertile independently of nuclear background or temperature. The results in *A. obtectus* suggest that the sex-specific effect of mitochondrial haplotype on fertility depends on complex interactions between the mitochondrial DNA, the nuclear DNA and the thermal environment.



Evaluating Wolbachia and host genetics for incompatible insect technique development in *Drosophila suzukii*

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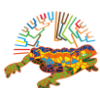
The incompatible insect technique (IIT) is a promising alternative strategy to broad-spectrum insecticides. It uses the bacteria *Wolbachia* to suppress pest populations in a more targeted and eco-friendly way. IIT is based on the phenomenon of cytoplasmic incompatibility (CI) manifested as embryonic lethality in crosses between *Wolbachia*-infected males and uninfected females. Understanding the factors that determine CI strength is important to develop IIT.

Drosophila suzukii, an invasive agricultural pest, causes major economic losses and is both a strong candidate for IIT-based control and a useful model for studying CI mechanisms.

We investigated the respective roles of *Wolbachia* and the host factors in driving CI

using *D. suzukii* strains previously shown to vary in CI strength (0-70% of CI). We found no effects of *Wolbachia* genotype on CI strength based on comparison of bacterial genomes associated with high and low CI. Furthermore, CI strength does not correlate with *Wolbachia* titers in testes. However, we found a great effect of the male host genotype on the strength of the phenotype and no effects of the female host genotype.

We emphasize that selecting a suitable host genotype is just as important as choosing the right *Wolbachia* strain. Potentially, the efficiency of incompatible insect techniques can be improved by using artificial selection of host genotypes to increase their susceptibility to *Wolbachia*-induced CI.



Gene drive dynamics in plants: the role of seedbanks

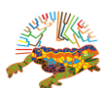
Kim I¹, Tian L^{1,2}, Chaffee R³, Haller B¹, Champer J⁴, Messer P¹, Kim J¹

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MEETING ROOM 118+119, August 18, 2025, 10:30 AM - 12:30 PM

Gene drives offer revolutionary potential for the management of problematic plant populations, such as invasive weeds and herbicide-resistant species, by rapidly spreading desired genetic alterations. Two recent studies have provided the first experimental demonstrations of engineered CRISPR gene drive systems in plants (CAIN and ClvR). However, the successful application of such systems in the field will critically depend on an accurate understanding of plant-specific life-history traits, especially seed dormancy, a ubiquitous yet frequently overlooked eco-evolutionary force. In this study, we develop the first comprehensive modeling framework for gene drives in plant populations that

incorporates a persistent soil seedbank. We show how the presence of a seedbank can significantly slow gene drive spread but also reduce the genetic load required to achieve population elimination. Furthermore, we show that seedbanks substantially increase the required introduction frequency of threshold-dependent gene drives, which could prevent establishment in some cases, yet also provide an intrinsic biosafety mechanism for confining a highly efficient drive to a target population. Our study highlights the need to incorporate seedbank dynamics into gene drive strategies to ensure more realistic predictions and successful field applications.



Mitigating resistance in gene drives using sex distortion strategies.

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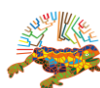
MEETING ROOM 118+119, August 18, 2025, 10:30 AM - 12:30 PM

Malaria remains a significant global health concern, with over 250 million cases and nearly 600,000 deaths reported worldwide in 2023. Children under the age of five are particularly vulnerable. The disease is transmitted by mosquitoes of the *Anopheles* genus, and current control measures—including vaccines and insecticides—have not proven sufficient to stop its spread. Gene drives are a promising biocontrol strategy that has the potential to significantly reduce the transmission of malaria. Genetic traits with super-Mendelian inheritance are introduced in the population enabling rapid genetic modification of the populations. However, a major limitation of gene drive technology is the evolution of resistance, which can restore population viability and undermine long-term effectiveness.

Recent studies have proposed integrating gene drives with sex distortion mechanisms, such as X-shredders, which bias offspring sex ratios toward males in drive-carrying individuals. In this work, we develop a spatially explicit stochastic model to investigate the dynamics of gene drive systems based on CRISPR-Cas9 technology

that incorporate sex distortion. We evaluate and compare the performance of various sex-distorter gene drive strategies under realistic ecological assumptions. Additionally, we contrast these with conventional single-guide RNA (gRNA) drives and multiplexed systems carrying two gRNAs, which have been proposed as a means to delay resistance.

Our results show that gene drives incorporating sex ratio distortion offer improved outcomes relative to single-gRNA designs, particularly in mitigating and delaying the spread of resistance alleles. Modelling suggests that sex distortion leads to enhanced performance in terms of probability of resistance as well as duration of the population suppression. These findings underscore the potential of sex-distorter gene drives as an effective and resilient tool for malaria vector control. Our modeling framework provides a flexible platform for evaluating drive performance under varying biological and environmental scenarios, contributing to the rational design of next-generation vector control strategies.



Out-of-locus genomic editors for efficient and localisable pest population suppression.

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MEETING ROOM 118+119, August 18, 2025, 10:30 AM - 12:30 PM

Effective control of pest populations remains a major challenge for agriculture, public health, and conservation. While currently utilised genetic control strategies—such as the release of sterile males or individuals carrying dominant lethal alleles (e.g., RIDL, fsRIDL)—have achieved some success, they often require repeated, large-scale releases, limiting scalability and applicability to species that are difficult to rear in the laboratory. Recent advances in CRISPR/Cas-based genome editing have enabled the development of more efficient genetic control methods that bias their own inheritance and impose a genetic load to achieve population suppression. However, these designs can spread throughout an entire species range, making them unsuitable when only local or temporary suppression is needed.

Here, we explore a class of self-limiting genetic control strategies that use genome

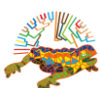
editors to increase the inheritance of deleterious alleles located elsewhere in the genome. The editor construct is inherited in a Mendelian fashion and does not drive itself, confining its impact in both space and time. Using a deterministic, discrete-generation simulation framework, we compare this strategy to existing approaches in terms of efficiency and temporal dynamics. Our results show that these editor-based strategies can achieve equivalent suppression while requiring the release of fewer modified males than traditional sterile insect techniques, with further gains achievable by targeting multiple sites or co-releasing a second construct that causes the first to transiently drive. We also propose feasible CRISPR-based molecular designs for implementing these systems. This work highlights promising alternative strategies for achieving localised, efficient, and reversible pest population suppression.





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S21 - Evolutionary consequences of heterokaryosis, mosaicisms, chimeras and other monsters



Heterosis in *Saccharomyces* hybrids: regulation of cold-adapted proteins under heat stress

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POSTER SESSION 1 - **P01.249**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: While interspecies hybridization is often an evolutionary dead end, certain interspecies hybrids display beneficial traits absent in either parent, a phenomenon known as heterosis. *S. cerevisiae* and the cold-adapted *S. uvarum*, can form viable hybrids despite distinct thermal limits. While *S. cerevisiae* grows up to 42°C and *S. uvarum* thrives at near-freezing temperatures, their hybrid tolerates both extremes, demonstrating heterosis. Although widely observed, the molecular basis that drives heterosis remains unknown.

This research investigates the fate of *S. uvarum* proteome in the hybrid under high temperatures, that are beyond its natural evolutionary range, exploring how it is regulated and whether it remains active in cellular processes. To reduce the complexity of the problem, we focus on a conserved cellular pathway: exocytosis, which is the secretion of cargo proteins to the plasma membrane and extracellular space. Exocytosis serves as an ideal model as it is essential for cell growth and requires regulation under changing temperatures.

Materials and methods: To investigate this, we employed a combination of genetic, imaging, and structural approaches to enable quantitative analysis of protein behaviors. Hybrid strains were constructed with fluorescently tagged exocytic proteins and allele-specific gene deletions. Live cell imaging with a precise temperature control system is used to track exocytic proteins at

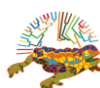
different temperatures and perform in vivo protein-protein interaction analysis. Transmission electron microscopy was used to assess cellular ultrastructure under heat stress.

Results: At 37°C, hybrids continued to express exocytic proteins from both the cold- and warm-adapted parental species, opening the possibility that survival at elevated temperatures involves functional contributions from both proteomes. Supporting this idea, the deletion of essential exocytic proteins from *S. cerevisiae* allele did not impair hybrid growth at 37°C.

Long-term live cell imaging at 37°C revealed that *S. uvarum* proteins initially formed intracellular aggregates; however, functional exocytosis later resumed, indicating a restoration of function.

Conclusions: Our findings suggest that hybrids actively stabilize and maintain the functionality of thermally sensitive proteins, highlighting regulatory mechanisms underlying heterosis at the protein level. Through detailed analysis of exocytic proteins, this project aims to elucidate the specific protein interactions and modifications that enable protein function to be maintained inside the hybrid cells.

The outcome of this research could have broader implications for understanding stress tolerance at the molecular level in other organisms and may enable biotechnological applications where temperature resistance is desired.



NLR evolution and the origins of heterokaryon incompatibility genes in *Podospora*

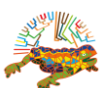
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POSTER SESSION 1 - **P01.247**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Early mycological studies on model fungi demonstrated that unrelated strains can fuse vegetatively into a syncytium of genetically different nuclei called a heterokaryon. However, in many species, fusion is halted by an incompatibility reaction, which likely functions to prevent resource plundering by conspecific nuclei and/or inhibit the spread of intracellular viruses and deleterious plasmids. Although heterokaryon incompatibility is widespread, the specific genes controlling rejection (het genes) vary greatly and are species-specific. Notably, multiple het genes do share a common domain architecture resembling NOD-like receptors (NLRs), intracellular proteins that detect pathogen infections in plants and animals. Moreover, all het genes evolve under balancing selection, as polymorphism serves as a proxy for self. Given these commonalities, what is the usual ancestral state of genes recruited for heterokaryon incompatibility? Here, we explore the evolution of NLR gene families related to four het genes of the ascomycete *Podospora anserina*. By characterizing their evolution within *P. anserina* and in comparison to other *Podospora* species, we show that NLRs undergo more rapid gene turnover than random genes, while having lower GC content, higher dN/dS values, and faster evolutionary rates. A subgroup of NLRs is distinguished by superstructure-forming repeats with very high sequence identity (high internal conservation, HIC), which

evolved independently multiple times. Unexpectedly, HIC NLRs are significantly associated with transposable elements, exhibit higher nucleotide diversity, and show elevated Tajima's D values indicative of balancing selection. Furthermore, unlike typical genes, HIC NLR phylogenies do not recapitulate species relationships, instead suggesting trans-species polymorphism as expected from long-term balancing selection. Yet, this observation requires precaution. Closer inspection revealed that NLRs are preferentially involved in introgression between *Podospora* species. Some introgressed NLRs have diversity patterns consistent with selective sweeps that deplete variation, while others are bi- or multiallelic. Additionally, we identified a case of repeat exchange between distinct HIC NLR paralogs, implying that novel binding specificities may evolve through repeat shuffling, thereby increasing allelic diversity. Overall, these findings suggest that NLRs, particularly HIC NLRs, evolve under diversity-enhancing mechanisms. In line with their general immune function in other eukaryotes, NLRs frequently display genomic signatures of both Red Queen dynamics and balancing selection, with or without introgression. Hence, heterokaryon incompatibility may readily evolve from such systems, co-opting pre-existing polymorphism essential for pathogen recognition into a novel conspecific self/non-self recognition trait.



Co-ordinated regulation of gene expression inside of dikaryotic Basidiomycetes

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MEETING ROOM 122+123, August 21, 2025, 2:00 PM - 4:00 PM

In Eukaryotes, mating generally occurs between two haploids leading to a diploid phase prior to meiosis. Basidiomycete fungi, particularly those that form mushrooms (Agaricomycetes), present an interesting exception to this pattern. In these fungi, mating between two haploid individuals occurs by the reciprocal donation of nuclei by both partners, followed by extremely rapid nuclear migration across both individuals. Instead of forming a diploid, a long lived dikaryotic stage with two haploid nuclei per cell is maintained. As each fertilising individual gains access to the resources accumulated by the other individual, a tension exists between the resident genome and the incoming, unrelated, fertilising genome. This tension may be increased by the occurrence of multiple, simultaneous, fertilising partners. Here we use RNA-seq of reciprocal matings between three wild isolates of the common wood-degrading fungus

Schizophyllum commune to ask if mechanisms have evolved in response to this tension, by modifying expression of genes from the fertilising, or the fertilised, nucleus. Utilizing the extremely high sequence divergence of *S. commune* we were able to distinguish expression of the alternate alleles of the vast majority (>99%) of genes. Our analyses recover significant numbers of differentially expressed genes depending on the monokaryotic or dikaryotic stage. Depending on the genetic background, there was strong correlation in the ratio of allele expression across life stages, indicating consistent dominant effects on gene expression. While there is significant evidence of differential expression within the dikaryons, we did not find a strong effect of mating role on subsequent expression. The results of this study indicate that haploid nuclei inside a basidiomycete dikaryon largely retain much of their individuality.



Fragmentation of mycelia caused loss of alleles and critical symbiotic traits in the domesticated fungus farmed by leafcutter ants

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MEETING ROOM 122+123, August 21, 2025, 2:00 PM - 4:00 PM

In heterokaryotic filamentous fungi, specific nucleotypes (genetically distinct nuclei) may become isolated within sections of the mycelium due to random segregation. Without mechanisms to promote the distribution of nuclei within the mycelium, heterokaryons would be evolutionarily unstable.

Leafcutter ants have obligately farmed a multinucleate fungal food crop (*Leucoagaricus gongylophorus*) for millions of years. The fungus exhibits key signatures of crop domestication, including the production of specialized swollen hyphal tips called gongylidia that ants eat, and exhibiting a type of polyploidy. While the ploidy of each nucleus remains uncertain, earlier research has shown: 1) that the fungus can lose alleles when isolated from ant farmers and regrown from hyphal fragments, and 2) that this loss of alleles can cause loss of gongylidia production. These studies indicate that the fungal cultivar is a heterokaryon containing different nucleotypes.

Leafcutter farmers appear to have solved these fragmentation issues since mature colonies can have millions of ants and hundreds of football-sized subterranean fungal chambers, each containing healthy fungus established from existing fungal fragments.

We first used microsatellites (10 loci) to link the allelic profile and gongylidia production in in vitro cultures undergoing experimental fragmentation. We then sampled mature leafcutter colonies in a Panamanian rainforest to test whether these massive colonies maintained the allelic profile across

spatially distant nest chambers. Finally, we used fluorescence live imaging to visualize whether and how individual hyphae can transfer nuclei through fusions (anastomosis) within the mycelium and between fungal fragments.

While fungi readily lost both alleles and gongylidia competency when passed five times in Petri dishes without ant farmers, the fungal cultivar maintained both of these traits when actively farmed by ants in the field. The in vitro fungi obtained significantly different allelic profiles after the sequential passing, whereas this was never observed between the fungal chambers in the colonies. Specifically, gongylidia-incompetent strains had an average of 70% fewer alleles than the initial parental strain, compared to an average of 15% of alleles lost between fungal chambers in all of the ten free-ranging Panama colonies. In vitro fungi further had only 1.3 alleles per locus compared to Panamanian fungi that had 2.6 alleles per locus. Furthermore, imaging enabled us to confirm the fungal-mediated processes of nuclear movement through anastomosis.

This study demonstrates that the loss of alleles and critical phenotypic structures can occur through the fragmentation and regrowth of mycelium. Notably, this phenomenon does not occur in nature. These results point to ant-farming strategies that complement fungal cellular adaptations for maintaining nucleotype diversity, thus promoting resilient farming in this iconic mutualism.



Nuclear cooperation as a driver of evolutionary transitions in fungi

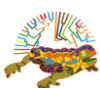
Siljestam M¹, Johannesson H¹

¹Stockholm University

MEETING ROOM 122+123, August 21, 2025, 2:00 PM - 4:00 PM

The evolution of complex life has been shaped by transitions in individuality, where distinct entities combine to form higher-level organisms. In fungi, heterokaryosis—a condition where genetically distinct nuclei coexist in the same cytoplasm—provides an ideal system to study such transitions. *Neurospora tetrasperma* is a filamentous fungus that transitioned from homokaryosis to heterokaryosis approximately one million

years ago. Using mathematical modelling within an evolutionary invasion analysis framework, we show that cooperation between two nuclear types drive this evolutionary shift. The study explore the conditions under which nuclear cooperation is favoured, providing insights into the mechanisms behind evolutionary transitions in individuality.



Premeiotic endoreplication as a compensatory mechanism in hybrid amniotes: A cellular pathway to asexuality, polyploidisation and genome stabilization

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¹Charles University, ²Institute of Animal Physiology and Genetics CAS

MEETING ROOM 122+123, August 21, 2025, 2:00 PM - 4:00 PM

Polyploidisation has played a crucial role in animal evolution, driving diversification in several major clades. Yet among extant amniotes, polyploidy is rare—and wherever it has become fixed, it is tightly associated with obligate parthenogenesis and hybridization. In most multicellular organisms, genetic homogeneity among nuclei ensures coordination and limits conflict. Yet in hybrid asexual vertebrates, the germline must manage profound intergenomic divergence between parental species. Our systematic comparative work of asexual vertebrates reveals a remarkably consistent cytological solution to this conflict. The overwhelming majority of obligate parthenogenetic and other asexual vertebrate hybrids employ premeiotic endoreplication, i.e. a duplication of the oogonia genome prior to meiosis, followed by a canonical meiotic process, including recombination. However, recombination occurs between sister chromatids, which typically preserves heterozygosity and maintains clonality. Nonetheless, we document that recombination contributes to structural genome reshuffling in some lineages, enabling genomic novelty even in this assumed clonal context. We confirm that over 20 independent evolutionary origins of asexual vertebrates involve premeiotic endoreplication, making it a recurrent cytogenetic strategy that facilitates asexuality in amniotes. This repeated co-option raises an intriguing question: why has this mechanism evolved so frequently? In asexual hybrids, over 95% of oocytes enter meiosis without prior endoreplication, fail to correctly pair divergent homologous chromosomes inherited from different parental species and undergo apoptosis. We propose that this massive

developmental failure triggers a compensatory mechanism, a polyploidisation in a subset of oocytes, leading to premeiotic genome duplication and restoration of homologous pairing potential, enabling meiotic progression. We hypothesize that premeiotic endoreplication functions as a compensatory response analogous to well-known adaptive somatic polyploidisation serving repair or stress response during healing and tissue regeneration. Under this view, the emergence of premeiotic endoreplication is a direct consequence of the failure to pair chromosomes in hybrid oocytes and the inherited repair compensatory mechanism connected with local polyploidisation. The mechanism of local polyploidisation in the germline of asexual hybrids not only stabilises asexuality, but it is a great preadaptation for organismal polyploidisation. The same cytogenetic strategy that allows unreduced egg formation in diploid parthenogenetic hybrids functions effectively in triploids as well. This enables incorporation of a haploid genome via sperm from a sexual relative. However, increased ploidy comes with costs: amniotes never exceed tetraploidy, and some lineages with premeiotic endoreplication do not even produce triploid lineages. We argue that germline polyploidisation in hybrid asexuals represents a key case of genetically composite development, where hybrid genomes—initially incompatible with normal meiosis—are stabilised through the recurrent co-option of ancient developmental programs. Premeiotic endoreplication deserves much greater attention in evolutionary biology as a major facilitator of genomic and reproductive innovations.

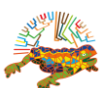




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S22 - Evolutionary Ecology of Microbial Symbioses



Bee gut bacteria and their arsenal: Evolution and Diversity of secretion systems

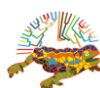
ACHEAMPONG S¹, Kwong W¹

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POSTER SESSION 2 - **P02.138**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The gut microbiota plays a crucial role in the physiology of the host. A stable microbiota, particularly its bacterial members, aids in nutrient metabolism, immune system modulation, and colonization resistance against pathogens. This stability is shaped by complex interactions among these members, which can be cooperative, such as metabolic cross-feeding, or antagonistic, such as competition for resources or ecological niches. A key mechanism proposed to mediate these interactions is bacterial secretion systems, specialized protein complexes that secrete effector molecules into neighboring cells or the surrounding environment to influence the stability of the microbial community. The bee gut harbors a simple and stable microbiota, which has been associated with certain secretion systems. However, the overall distribution of these systems, their association with different bee species, and their evolution have not been fully explored. Here, we show that bee gut bacteria have evolved to possess secretion systems that specialize in colonization and interbacterial competition rather than in host antagonism. Using a comparative genome analysis approach that compares the proteins of conserved components, we found that bee gut symbionts predominantly encode type V (T5SS) and type VI (T6SS) secretion systems. In contrast, the typical virulent type II (T2SS) and type III (T3SS) systems are replaced by

evolutionarily related appendages, flagella and pili, for colonization. Interestingly, we found little to no association between the presence of specific secretion systems and the bee hosts, suggesting that these systems are maintained primarily for interbacterial interactions rather than host-specific adaptation. Global-fit co-phylogenetic analyses, supported by permutation-based statistical testing, indicate significant congruence between bacterial phylogeny and secretion system phylogeny, suggesting a vertical mode of transmission for the secretion systems. Furthermore, phylogenetic analyses indicate that the evolutionary history of these systems is characterized predominantly by loss events. Our results suggest that the evolution of secretion systems in bee gut symbionts may be driven more by microbial community interactions than by adaptation to specific bee hosts, which in turn contributes to shaping a stable microbiota. We anticipate that understanding how fundamental cellular mechanisms like secretion systems evolve provides insight into the driving factors governing gut microbiota stability. Our results provide a framework for experimentally manipulating the microbiota to explore how interactions contribute to stability, as a proxy to further elucidate their role in host physiology.



Beyond abundance: Symbiont transcriptional plasticity meets host's nutritional requirements

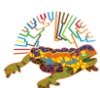
Ayas A¹, Salem H¹, Pons I¹

¹Max Planck Institute for Biology, Tübingen

POSTER SESSION 2 - **P02.124**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Quantifying how symbionts are regulated throughout host development provides key insights into the trade-offs shaping beneficial partnerships. We explore this dynamic in the digestive symbiosis between tortoise leaf beetles (Coleoptera: Cassidinae) and *Candidatus Stammera capleta*, a gammaproteobacterium. Tortoise beetles harbor *Stammera* extracellularly within foregut symbiotic organs to facilitate folivory and in ovary-associated glands to ensure vertical transmission. *Stammera* possesses a highly reduced genome (~0.25 Mb), where it encodes plant cell wall-degrading enzymes on a plasmid. Given the vital role *Stammera* plays in upgrading host digestion of plant material, we investigated the microbe's population dynamics in relation to folivory and stage-specific nutritional requirements. Folivory was quantified as a proxy to assess the beetle's dietary need and growth across developmental stages. Our findings reveal that foliage

consumption is correlated with shifts in host development, increasing significantly over time. Strikingly, *Stammera* abundance remained stable within the foregut symbiotic organs throughout development, despite a rapid increase in its host's overall weight and size as larvae progressed to adulthood. However, the plasmid copy number, and the expression of *Stammera*-encoded plant cell wall-degrading enzymes, were dynamically regulated to meet the dietary needs of a host contending with greater amounts of leaf material. Collectively, our findings demonstrate that transcriptional plasticity, rather than *Stammera* population size, mediates symbiont contributions to host physiology. Our findings highlight how symbiosis supports stage-specific nutritional and physiological needs, providing insights into the molecular and evolutionary dynamics of host-symbiont interactions, co-evolution, and adaptation.



Breaking eco-evolutionary feedbacks leads to extinction in a bacterial mutualism

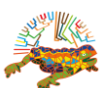
Baranovitch Y¹

¹The Hebrew University of Jerusalem

POSTER SESSION 2 - **P02.142**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Coevolution is a key force structuring how ecological communities form and function. Specifically, eco-evolutionary feedbacks (EEFs), consisting of reciprocal adaptations between interacting species, are thought to be a key mechanism driving coevolution within communities. EEFs have been shown to be crucial in antagonistic interactions, where they lead to evolutionary arms races. However, the effect of EEFs on other types of interactions is poorly understood. In particular, it is unclear whether EEFs reinforce or degrade mutualistic interactions, which are widespread interactions shaping ecological communities. To address this knowledge gap I developed an experimental system that allows evolving one species in the presence

of another species that is maintained in its ancestral state, preventing any EEFs. Using this system, I (co)evolved a pair of *Escherichia coli* strains that exchange essential amino acids and form an obligate mutualism. Surprisingly, preventing EEFs destabilized our system, and both species survived only when they coevolved. The survival was driven by the evolution of one of the species that evolved to higher density, reducing its amino acid requirements for growth and thereby supporting its partner's survival. These findings reveal a case in which the evolution of one species plays a key role in preventing the extinction of its partner, underscoring the intricate dynamics of coevolution within microbial communities.



Characterization of *Klebsiella pneumoniae*-Targeting Phages in an In Vitro FUT2-Dependent Mucosal Model: Implications for Host-Phage Evolution

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POSTER SESSION 2 - **P02.145**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: The human FUT2 gene encodes an α 1,2-fucosyltransferase responsible for attaching fucose to glycan chains on mucosal surfaces, thus determining the glycosylation profile of mucus (1). This modification shapes the intestinal microbiome by influencing composition and localization of various microbes, including the opportunistic pathogen *Klebsiella pneumoniae* (Kp) (2). Individuals with a functional FUT2 allele are considered “secretors,” while those homozygous for loss-of-function mutations are classified as “non-secretors”, comprising approximately 20% of individuals in European populations (1). Bacteriophages, which specifically infect and lyse bacteria, may also interact with host mucus glycans in order to enhance retention and antibacterial activity, as proposed by the Bacteriophage adherence to mucus (BAM) model (3). Considering the impact of FUT2 genotype on terminal glycoprotein fucosylation, it may represent a critical host genetic factor to be considered in personalized microbiome-based phage therapies.

Materials and methods: A panel of lytic phages targeting *Klebsiella pneumoniae* strains was characterized through genetic analysis using bioinformatic tools. Host range was assessed in both solid and liquid media. Phage-mucin interactions were evaluated using adhesion and efficiency assays. Mucin preparations included untreated mucin containing terminal fucosylated glycans (representing FUT2 secretor-type mucin) and mucin pretreated with α 1-2-fucosidase to remove terminal fucose residues (representing FUT2 non-secretor-type mucin). In order to specifically investigate the role of fucose in phage-mucin interactions, a competitive binding assay was performed by pre-incubating phages with L-fucose prior to testing in the presence of mucin.

Results: Comparative genome analysis of Kp-specific phages revealed considerable variation in genome size and coding capacity. Host range testing revealed differential lytic profiles across

environmental and antibiotic-resistant, clinical Kp strains. Two phages, classified into the Straboviridae family, harboured a gene homologous to the highly immunogenic outer capsid (Hoc) protein coding sequence, which has previously been associated with mucin glycan binding in T4-like phages (3). Both the predicted protein structure and the immediate genetic context of the Hoc-like gene are similar to those of T4-like phages. Preliminary observations indicate that mucus may enhance phage adhesion on mucins and phage lytic activity against Kp strains. Ongoing assays aim to determine whether terminal fucosylated glycans, shaped by FUT2-dependent mucosal fucosylation, are a critical factor mediating phage-mucus interactions and enhancing lytic efficacy.

Conclusion: Certain phages, especially T4-similar ones, encode Hoc-like proteins that may facilitate interactions with host intestinal mucus. Ongoing work will assess the role of terminal fucosylated glycans, dependent on the host's FUT2 gene status, in influencing phage localization and lytic efficiency. These findings aim to highlight the importance of considering host genetics and host-phage co-evolution in the development of personalized microbiome-modulating therapeutic strategies against opportunistic pathogens like Kp.

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2 Cheng S, Hu J, Wu X, et al. Altered gut microbiome in FUT2 loss-of-function mutants in support of personalized medicine for inflammatory bowel diseases. *Journal of Genetics and Genomics.* 2021;48(9):771-780. doi:10.1016/j.jgg.2021.08.003.

3 Barr JJ, Auro R, Furlan M, et al. Bacteriophage adhering to mucus provide a non-host-derived immunity. *Proceedings of the National Academy of Sciences of the United States of America.* 2013;110(26):10771-10776. doi:10.1073/pnas.1305923110.



Composition of gut microbiota is mostly driven by developmental stage and substrate in an invasive insect pest

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POSTER SESSION 2 - P02.148, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

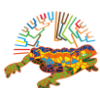
Over the last decade, many studies have demonstrated that the gut microbiota strongly affects essential traits associated with fitness, such as immunity or growth. For example, axenic *Drosophila* larvae (without any microbiota) cannot develop and die at an early developmental stage. Studies has also shown that the gut microbial community is shaped by the host. However, the relative role of the host life stage, fruit substrate, and host genotype on the composition of the bacterial and fungal gut communities has been less studied.

Here we study how these three factors affect the bacterial and yeast communities composition of *Drosophila suzukii*, an exotic invasive fruit pest that infests multiple host plants.

To test for the potential effects of the host life stage, fruit substrate and host genotype on the gut bacterial and yeast communities of *D. suzukii*, we used a metabarcoding approach by sequencing 16S and ITS

markers. We artificially infested fresh fruits (strawberry, cherry and blackberry) with lab-reared flies (two different genotypes). We extracted larvae from host fruits and transfer them to a pasteurized medium until they pupate. We sampled individuals at each developmental stages (larva, pupa, adult) and fruits before and after infestation.

Our first results indicate that the yeast community is strongly structured among life stages (i.e. larvae, pupae, adults) and among fruit varieties (i.e. strawberry, cherry, blackberry), but not among host genotypes. On the contrary, the bacterial community is shaped mostly by life stages. These preliminary results indicate that metamorphosis plays a crucial role in shaping the gut microbial community. We will discuss how different transmission patterns between bacteria and yeast communities may affect the fitness of insect hosts.



Contextualizing symbiosis: microbial interactions, host environments, and the dynamics of association

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POSTER SESSION 2 - **P02.136**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

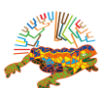
Symbioses are typically defined as persistent interactions between species, yet the ecological and evolutionary dynamics underpinning these associations are far from fixed. Mounting evidence indicates that the outcomes of microbial interactions—both among microbes and with their hosts—vary widely in their context-dependency. In this talk, I explore the variability in microbial interaction outcomes and how it influences the stability, adaptability, and evolutionary potential of symbioses.

Drawing on empirical work on microbial predation (Vasse et al., 2024, PLOS Biology), I show that even interactions as seemingly directional as predation can reverse under certain ecological conditions, challenging assumptions of fixed roles within microbial communities. In a study of the human vaginal microbiota, we found that the metabolic activity of individual strains is strongly modulated by the presence of other strains or species. These findings underscore that microbial functionality—and, by extension,

effects on the host—cannot necessarily be predicted from isolated taxa alone.

Building on these findings, I discuss a conceptual framework that incorporates a spectrum of ecological context-dependency within symbiosis. This framework considers how host traits—such as immune filtering, niche provisioning, or spatial structuring—can influence whether microbial communities experience more stable or more variable environments, with implications for predictability and evolutionary outcomes.

This work aims to enrich our understanding of symbiosis as a dynamic process mediated by fluctuating ecological conditions and multi-scale interactions. I argue that incorporating interaction variability into our models of symbiosis can better capture the diversity of host-microbe systems and help explain how microbial symbioses function as both stabilizing forces and engines of evolutionary innovation.



Disentangling Ecological Variability: The Role of Richness and Environment in Gut Microbiota Dynamics

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POSTER SESSION 2 - P02.153, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

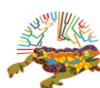
Understanding the drivers of ecological community variability remains one of ecology's fundamental challenges. Communities in seemingly similar environments often display diverse compositions, yet alike communities can sometimes be found in highly dissimilar environments, patterns that defy straightforward predictions. This unpredictability appears to increase with community complexity, suggesting that ecological interactions play a crucial role in shaping community assemblages. The interplay between biotic and abiotic factors further complicates our understanding of system dynamics, raising important questions about the nature of cross-system variability. In this context, the integration of theoretical frameworks with empirical findings has been crucial to shed light on these intricate scenarios, a strategy that has been adopted in our work.

In this study, we investigate *in vivo* gut microbiota compositions, using two distinct mouse models exposed to varying environmental conditions, to determine to what extent community dynamics follow deterministic patterns. The first mouse model features a conventional microbiota of specific-pathogen-free (SPF) laboratory mice, characterized by high richness and diversity, while the second system consists of a gnotobiotic mouse model colonized with a defined synthetic community of 12 bacterial species, known as the Oligo-Mouse-

Microbiota-12 (OMM12)*. To experimentally manipulate the environment of SPF and OMM12-colonized mice, considering the impact of nutrient availability on the gut microbiome, mice were treated with different diets. By focusing on reproducibility and the relationship between species' average abundance and variability, our research specifically examines how species richness and environmental conditions influence these dynamics.

By analyzing patterns across varying levels of community complexity and diets, we demonstrate that variability is strongly affected by species richness. Fluctuations, which are mostly proportional to abundance when many species coexist (Taylor's law), become sublinear with respect to abundances at low richnesses. Furthermore, to mechanistically understand these systems, we analyze fluctuations in the functional potential of communities to respond to perturbations caused by different dietary treatments, attempting to unravel a possible hierarchy between taxonomic and functional compositions of bacterial communities. We expect that integrating ecological theory with our experimental results will not only characterize the differential ecological principles governing each community but also reveal the biological implications of microbiota functions in response to different diets and their effects on the host.

* Brugioux, S., et al. (2016). *Nat. Microbiol.* 2, 16215.



Eco-evolutionary dynamics of Pisum-Bacteria interactions mediated by reciprocal selection

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POSTER SESSION 2 - **P02.125**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Plant-microbiota interactions play a key role in plant adaptation by improving plant nutrient acquisition, abiotic and biotic stress resilience, and overall fitness. While these interactions are receiving growing attention in agriculture and conservation efforts, the ecological and evolutionary processes governing the assembly and function of plant-associated microbial communities are poorly understood. Emerging evidence indicates that these associations may be shaped by co-evolutionary processes whereby host evolutionary history explain its microbiota composition and function. However, these evidences remain limited with much of the recent work focusing on phylosymbiosis. While phylosymbiosis can reflect the influence of host phylogeny on the ecological structure of associated microbiota, it is essential to determine whether it also shapes the phylogenetic structure of the host-associated microbial community and whether closely related hosts associate with closely related microorganisms. Such co-phylogenetic patterns would inform to which extend the plant-microbiota interactions have driven the diversification and adaptation of both partners. We conducted a greenhouse experiment with 24 *Pisum* spp. accessions, comprising a total of 240 individual plants, to study plant-bacteria interactions across different plant-associated microhabitats. Bacterial DNA was extracted from bulk soil, rhizosphere, rhizoplane, endosphere and nodules microhabitats, resulting in the characterization of over 800 bacterial communities with full-length 16SrRNA gene sequencing. Through an integrative framework combining plant and bacterial

phylogenetics, bacterial community ecology, bacterial functional traits and plant growth kinetics, we tested whether these interactions may reflect pairwise coevolution. We show that bacterial communities significantly differ across *Pisum* genotypes and reflect phylosymbiosis, with closely related hosts hosting more similar communities. Furthermore, phylogenetic structure of bacterial communities across host genotypes and plant-associated microhabitats reveals significant clustering, suggesting that plant-bacteria associations selectively favour specific bacterial lineages. Shifts in bacterial community composition and phylogenetic structure were coupled with changes in bacterial functional gene expressions, especially in nitrogen cycling, highlighting that host-driven filtering of microbial taxa may modulate ecosystem functions. Given that host phylogeny best explained the variation in plant developmental rate, we evaluate the degree to which this plant-specific trait relates to the structure and function of bacterial communities. Altogether, these findings suggest that both the host plants and their associated microbial communities act as niche constructors, with reciprocal effects on each other's fitness as reflected in the evolutionary patterns of their interactions. This study significantly enhances our understanding of plant-bacteria interactions by integrating ecological, evolutionary and functional perspectives, and opens new avenues for investigating their coevolution with potential implications for plant adaptation and microbiome-based innovations.



Eco-evolutionary feedbacks in *C. elegans*-microbe interactions link community dynamics to genetic evolution and back

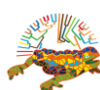
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¹University Of Amsterdam, ² KU Leuven Kulak

POSTER SESSION 2 - **P02.146**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Ecological and evolutionary trajectories of animals and their symbiotic microbes are co-dependent and their interaction is a rich source of adaptive potential. This is illustrated by widespread patterns of phyllosymbiosis and evidence for host-microbe co-speciation as a major predictor for global and local patterns of species diversity. In a series of experiments using the model nematode *Caenorhabditis elegans* and naturally co-occurring microbes, we explored: 1) how host genetics and host behavior shape microbiome assembly; 2) how microbiomes can contribute to evolutionary dynamics in the nematodes; and 3) how nematode evolution feeds back on the assembly of symbiont microbial communities. Nematodes live in bacteria-rich environments and engage with bacteria in a broad range of ecological interactions. Studying nematode-bacteria interactions is fundamentally important for understanding nematode ecology and evolution and the evolutionary ecology of species interactions more generally. In a first experiment, *C. elegans* evolved for 20 generations in the presence of a novel dietary bacterium. The experiment was conducted at five different research institute across the Netherlands and Belgium. Lab-specific environmental bacteria that were sequenced along with the worms were found to influence fitness evolution and the repeatability of evolutionary change. Looking at changes in the *C. elegans* genomes during experimental evolution, we identified candidate genes underpinning adaptive evolution in the microbially diverse environments. Microbial

assembly experiments with knock-out lines for these candidate genes confirmed that these genes functionally affect community assembly in the nematodes. In a second experiment, *C. elegans* evolved for 12 generations under high versus normal salt conditions with and without a gut microbiome. We found that evolving with a gut microbiome facilitated an early evolutionary response to high salt. Evolving in the presence of high salinity modified the gut microbiome assembly of the nematodes. Intriguingly, we found the relative abundance of bacteria known to be involved in salt stress responses in plant root systems to be higher in the nematodes evolving in high salt environments compared to those evolving in unmodified environments. While this can be explained in part by higher fitness of salt tolerant bacteria in saline environments, we also hypothesize that behavior of the nematodes may play a role. In a series of choice experiments we reveal that environmental context drives bacterial preferences in the nematodes, lending support to the host behavior-dependent assembly hypothesis. Together, these results illustrate the intricate relationship between host ecology and evolution and symbiont community composition. By combining Evolve and Resequencing approaches with microbiome assembly experiments in knock-out lines, we begin to uncover genetic mechanisms mediating ecological interactions that feedback onto evolutionary dynamics of host and microbes.



Evolution of gut microbiome during ageing in short-lived African turquoise killifish

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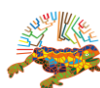
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POSTER SESSION 2 - P02.143, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Commensal microbes play key roles in multicellular host development, survival, and disease susceptibility. Throughout an organism's lifespan, the composition and metabolic function of microbial communities within the gut undergo substantial shifts, influencing critical phenotypic traits such as nutrient absorption, vitamin synthesis, and protection against pathogens. Despite accumulating evidence of the dynamic evolution of the microbiome within the host, a comprehensive understanding of the ecological and evolutionary processes shaping microbial dynamics over the host's lifespan remains limited.

In this study, we aim to investigate the temporal patterns and mechanisms driving

age-related alterations in gut microbial communities, as well as their potential impacts on host health across different life stages. Leveraging the naturally short lifespan of the African turquoise killifish (*Nothobranchius furzeri*) as a model organism, which possesses a diverse gut microbiota, we aim to characterise the compositional and functional shifts in gut microbiome throughout the ageing process. Our research will explore the interplay between host ageing and microbial dynamics. Ultimately, this work seeks to provide novel insights into the ecological and evolutionary dynamics of host–microbe interactions during ageing, with potential implications for microbiome-based strategies to promote healthy ageing.



Expanding the *P. bursaria*-algal model for endosymbiosis

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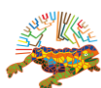
POSTER SESSION 2 - **P02.133**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The living of one organism within another, endosymbiosis, has evolved independently multiple times across the tree of life and often includes interactions between vastly unrelated organisms. This phenomenon is central to our understanding of the evolution of emergent complexity in modern life and forms a cornerstone in our understanding of ecology, sustainable agriculture and, increasingly, medicine. In natural settings endosymbioses typically occur in the context of complex and diverse ecosystems, however, when studied they are often reduced to one-on-one interactions between two organisms. This reductionist approach risks obscuring important dynamics and evolutionary pressures that emerge in diverse communities. For further mechanistic and evolutionary understanding of endosymbiosis, model systems that incorporate ecological complexity are needed.

Paramecium bursaria has emerged as a model for the study of primary and secondary endosymbiosis in eukaryotes. It engages in a facultative symbiosis with several species of green algae which are housed in specialised vacuoles that mediate the transfer of amino acids and carbon dioxide to the symbiont in exchange for oxygen and photosynthetic products. The host can exert strict control over its symbiont load, optimising it for carbon output per unit nitrogen cost. *P. bursaria* can hold multiple algal species simultaneously, a feature that is yet to be explored experimentally. We propose expanding the *P. bursaria*-algae model to include the study of these naturally occurring tri-partite systems whereby *P. bursaria* plays host simultaneously to microalgae from the genera *Chlorella* or *Micractinium* and picoalgae in the genus *Choricystis* in what we described as dual-algal systems.

To develop this model we tested the viability of 15 different *Choricystis* strains, isolated from free-living and symbiotic populations, to act as symbionts for *P. bursaria* (186b). These strains were tested in cured hosts either as the sole symbiont or in combinations with the host's native symbiont *M. conductrix*. Populations were monitored via flowcytometry, manual counts and fluorescence microscopy. Though all strains were ingested by the host and held in digestive vacuoles, their ability to incorporate and persist in the host's cortex as symbionts varied greatly. Two symbiotic strains (s171 and s160) were capable of sustaining hosts for 6 weeks as the sole symbiont. However, in combination with *M. conductrix*, the native symbiont came to fixation in the long term in most instances. Despite this, many strains of *Choricystis* can cohabit the host's cortex for up to two weeks, while others showed signs of longer-term stability (further investigation is needed for confirmation). These data suggest a window of opportunity for short- and medium-term experimentation to take place.

These findings allow for a more ecologically relevant model system in which questions of host control, symbiont compatibility, and evolutionary trajectories can be examined in a tripartite system. Our next line of investigation is to test the fitness outcomes of hosts infected with *Choricystis* alone, *M. conductrix* alone and a combination of the two along a light gradient to determine host control over the novel symbiont load, and fitness outcomes for all involved. This allows us to gain novel insights into how novel symbioses can be formed and stabilised in diverse ecosystems. By increasing the number of selfish entities that simultaneously co-habit the host, we hope complexity seen in nature can be mirrored in a tractable system.



Experimental evolution within the bee gut

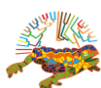
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POSTER SESSION 2 - **P02.139**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Many animals have successfully expanded into a range of ecological niches in part due to the benefits conferred by their microbial symbionts. These beneficial symbionts may provide essential nutrients, defence against antagonists, or protection from abiotic stressors. While studies have explored the functional benefits of host – microbial symbioses, how symbionts establish themselves in a novel host and the how partner – specificity evolves is less known. From the microbial perspective, we hypothesize that differences in strain origins, growth conditions and interactions with other community members influence their evolution and establishment within the host. Experiments with a tractable symbiotic system where we can perform host – switching and experimental evolution in vivo will help us observe the genomic changes that occur during host – specialisation and understand the factors that influence within – host symbiont evolution. For this, social bees and their well-defined set of beneficial gut microorganisms comprising mainly of *Snodgrassella*, *Gilliamella*, *Lactobacillus*, *Bombilactobacillus*, *Bifidobacterium*, are a simplified and experimentally tractable model system. Bee gut microorganisms help in breaking down complex plant substrates to provide nutrients and also provide colonisation resistance. Although the core microbial species associated with the host are consistent across eusocial corbiculate bees, studies show that, at the strain level,

the symbionts exhibit high diversity and host – specialisation. Eg. Strains of *Snodgrassella* isolated from bumble bees, do not effectively colonise honey bees and vice versa. What led to strain specialisation in the bees and can a non – native strain adapt to the bee gut through frequent exposure? To study this, native honey bee gut microbial strains (*Snodgrassella alvi* KFP26 and wkB2) and a non – native bumblebee gut microbial strain (*Snodgrassella communis* wkB12) were introduced to honey bees. Monocultures of the strains were fed to honey bees in a cup cage. Five days post-inoculation, gut contents of the colonised bees were fed to a new group of newly emerged bees. At the end of each passage, the CFU counts of each strain in the honey bee guts were estimated. After six passages, we enrich for bacterial cells from individual bee guts and extract bacterial DNA for shotgun metagenome sequencing to identify variants in the evolved population. We observe that the native and non-native strains always colonise ~100% and ~50% of the bees in each passage. In the evolved strains, we aim to pinpoint microbial genes or loci that are under selection and understand how it affects strain adaptation in vivo. Ultimately, through within – host evolution experiments in the presence of the rest of the gut microbial community we wish to enhance our understanding of microbial evolution and factors that lead to host – specialisation in bee gut bacteria.



Exploring the evolutionary ecology of microbes associated with the common garden snail

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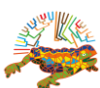
POSTER SESSION 2 - **P02.147**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The common garden snail *Cornu aspersum* is a cosmopolitan agricultural pest. A snail's body wall presents to its environment a mucosa rich in specialised glandular cells, which are believed to perform essential nutritional and defensive functions. Though overlooked, this mucosa plays a central role mediating microbial interactions. Here we explore *C. aspersum*'s cuticular microbiome.

Snails sampled across Europe were characterised by nanopore 16S rRNA sequencing mucus, revealing a microbiota rich in *Erwinia* sp. and *Pseudomonas* sp. Confocal fluorescent microscopy confirmed that bacterial symbionts are resident in cuticular bacteriocytes, and especially abundant in the anterior portion of the foot

and in the mantle. Symbionts could be cured using high doses of antibiotics, but this was associated with high snail mortality. Provision of antibiotic-resistant *Pseudomonas* symbionts rescued hosts from death, however the extent of colonisation was negatively correlated with host growth in surviving individuals. Using RFP-transformed antibiotic-resistant *Pseudomonas* to visualize invasion, we observed competitive exclusion by resident strains even under strong antibiotic selection favouring the invader.

Our results suggest that symbiosis in *Cornu aspersum* is widespread, primarily based on diverse strains of *Pseudomonas*, and that one of its likely functions is mucosal immunity.



Foregut microbial communities and the possibility of endogenous ethanol production in honeybees

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POSTER SESSION 2 - **P02.128**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Honeybees encounter low doses of ethanol in the environment, primarily in fermenting nectar, which can have both negative and positive impact on their physiology. However, we recently detected traces of ethanol in the crop (part of the foregut) contents of caged bees maintained without access to environmental food sources, raising the possibility of endogenous ethanol production mediated by the microbiota. While the honeybee gut microbiota is known to be composed of a small number of 'core' species typically concentrated in the hindgut, the crop is considered particularly poor in bacteria. At the same time, the primary function of the crop is to store and transport the ingested food. We hypothesized that shifts in the crop microbiota under laboratory conditions may facilitate fermentation, potentially contributing to endogenous ethanol accumulation independent of environmental exposure.

Materials and methods: We analyzed ethanol levels in crop contents (n = 207, 3 µl per sample) from two groups of the same age: caged bees fed sucrose solution and naturally foraging hive bees. Ethanol concentrations were quantified using a spectrophotometric assay. To characterize microbial communities, pooled crop contents were subjected to 16S rRNA (V3-V4 region) metabarcoding, resulting in 11 and 12 group-level samples. Amplicon sequence variants (ASVs) were assigned to taxonomic units using the BEEexact database, specific to the bee microbiome. Microbial communities were compared using α- and β-diversity metrics, DESeq2 differential abundance testing, constrained correspondence analysis (CCA), and comparisons of within-group divergence and generalized linear mixed models (glmmTMB) focusing on key genera.

Results: Mean ethanol concentrations in both groups were low and near the method's detection threshold (~0.012 g/l in hive bees,

~0.007 g/l in caged bees), but non-zero measurements were more frequent in caged bees. Microbial community structure differed strongly between groups: caged bees hosted significantly fewer ASVs (981 vs. 2,358 in hive bees), with only 7% of variants shared. At the genus level, diversity was also reduced, but β-diversity was higher among caged bees, indicating greater within-group divergence. In caged bees, the abundance of genera connected with flower nectar (like *Apilactobacillus*), which constituted the majority of hive bees' crop microbiota, was significantly decreased. Several genera were enriched in caged bees, three of which represent the bees' core microbiota, including *Gilliamella*, capable of producing ethanol. This indicated the potential to acquire ethanol not only from the environment but also from metabolic processes performed by bees' microbiome.

Conclusions: Our findings suggest that under constrained dietary and environmental conditions, crop-associated microbial communities may acquire novel, or enhance existing, metabolic functions, including the ability to ferment sugars into ethanol. These changes include a loss of microbial diversity and a shift in community composition, at least in part toward fermentation-capable taxa. Our study highlights the dynamic nature of host-microbe interactions in honeybees and raises the possibility that the bee microbiota contributes to modulation of endogenous ethanol levels, with potential consequences for host physiology and behavior. The extent of these consequences remains to be studied, but our results already underscore the evolutionary and ecological significance of microbial symbioses as sources of phenotypic plasticity in host species.

This research was funded by the National Science Center in Poland, Sonata 17 grant 2021/43/D/NZ8/01044.



Host-gut microbiota coevolution in insular populations: A case study of the Balearic lizard *Podarcis lilfordi*

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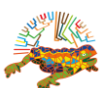
POSTER SESSION 2 - P02.132, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Insular populations, due to their isolation, provide a great natural system to explore evolutionary processes between vertebrates and their gut microbes. Through population-level studies, they allow us to investigate critical questions such as how host genetics affect gut microbial community structure.

To address this, we studied multiple populations of the endemic Balearic lizard *Podarcis lilfordi*. Using full-length 16S rRNA sequences (Oxford Nanopore Technology) from fecal samples of 240 individuals across 11 islets, we generated gut microbial profiles and coupled them with host population genetic distances based on the fixation index (FST) and single-nucleotide polymorphisms (SNPs). To minimize the influence of extrinsic factors in shaping gut microbiota composition, we focused only on bacterial taxa present in at

least 80% of individuals within each population (i.e., the core 80%).

All populations showed a remarkably conserved core gut microbiota taxonomic profile, despite having been isolated for 4,000–9,000 years, suggesting ancestral inheritance and a strong resilience of these bacterial communities. Relative distances among populations inferred from gut microbiota closely mirrored those based on host population genetics, pointing to an influence of the host phylogeographic history on gut microbial composition. Additionally, we identified a set of bacterial genera responsible for this pattern, which likely represent the “housekeeping” component of the lizard gut microbiota. These taxa emerge as important candidates for future studies aimed at better understanding host-microbe coevolution and its role in insular adaptation.



Investigating Past Human Mobility and Interactions in the Southern Cone of the Americas using Phylogenomics of Ancient Commensal Microbes.

Willingham Grijalba A¹, Ponce-Soto G¹, Fumey J¹, Lopopolo M¹, Nelson E¹, Tressieres G², Iraeta-Orbegozo M³, Kabral H⁴, Peralta E⁹, Abbona C⁹, Lucero E¹¹, Recalde A¹², Díaz I⁸, Rivero D¹², Guevara D¹³, Da Peña G¹³, Orlando L², Schroeder H³, Scheib C⁴, Méndez C⁵, Nuevo-Delaunay A⁶, Luna L⁷, Aranda C⁸, Ratto N⁸, Scartascini F¹⁴, Borella F¹⁵, Berón M¹¹, Goñi R⁸, Tessone A¹⁶, Garcia Guraieb S¹⁷, Pastor S¹⁸, Tissera L¹⁹, Barberena R²⁰, Novellino P¹³, Neme G⁹, Gil A⁹, Del Papa M¹⁰, Rascovan N¹

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POSTER SESSION 2 - P02.120, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Dental calculus, the calcified biofilm that accumulates on teeth's surfaces during an individual's life, preserves an exceptional archaeogenetic record of hundreds of microbial species that are part of the human oral microbiome. However, most of what we know about oral microbiomes is based on modern samples, and little is known about how these human-microbe associations were established over large spatiotemporal scales. Previous metagenomics works –on modern and ancient samples– have found relatively low differences in the oral microbiome composition of populations from different periods and continents. Thus, we hypothesised that if oral microbial species are maintained within human populations over long periods, their genomes could reflect human population movements through time. To test this hypothesis, we sequenced and analysed ancient dental calculus samples from multiple individuals from

the Southern Cone of the Americas, a region with a rich archaeological record, but underexplored using paleogenomic approaches. Phylogenomic methods were used to reconstruct the relationship between strains of oral microbial species recovered from individuals across multiple spatiotemporal contexts. When combined with archaeological data, we revealed several species with strong spatiotemporal associations with their host populations. Together, our results bring not only unprecedented information about the oral microbial diversity associated with indigenous populations of South America but also novel insight into the long-term association between humans and their commensal microbes. We propose that this type of data and the implemented analytical approaches could be also used as a novel and complementary approach to investigate the mobility and interactions of ancient human populations.



Investigating signatures of local adaptation in the ubiquitous forest ectomycorrhizal fungus *Cenococcum geophilum*

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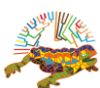
POSTER SESSION 2 - P02.123, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Rising temperature- and drought-induced stress on European forests has increased tree mortality, yet little is known about the genetic mechanisms of climate adaptation in their associated ectomycorrhizal fungi and the role of a common evolutionary history. We focus on the symbiotic interaction of Pedunculate oak (*Quercus robur*), a ecologically and economically important forest tree species, and *Cenococcum geophilum*, a widespread, drought-resistant ectomycorrhizal fungus with a broad host range, often dominant in temperate and boreal forest ecosystems.

Materials/Methods: Using in situ soil measurements and modeled atmospheric data across 21 sites within the range of the host tree, *Quercus robur*, we isolated fungal strains from three soil depths per site. We will conduct whole genome sequencing of individual strains to identify genotype-environment associations and pinpoint genomic regions underlying local adaptation, especially selective pressures associated to biotic and abiotic factors. Additionally, we will genotype 16 oaks per site to investigate population structure and the shared demographic history of this host-fungus pair. Finally, we will identify genotype-genotype co-occurrences in host and symbiotic partner that are associated with certain environmental conditions.

Results: We expect to find various *C. geophilum* lineages within and across sampling sites and depths, with specific gene variants to be associated with soil chemistry and water availability. In addition, we anticipate complex gene pathways involved in adaptation to local dry conditions with functions related to melanin production and other desiccation-resistance traits. We expect *Q. robur* populations to be mainly structured along a latitudinal gradient, by topography, and other persistent barriers. Also, we expect to find varying admixture levels of individuals within and between populations. Finally, we expect to identify genotype-genotype combinations that are associated with certain climatic and soil conditions and to identify coincidences of effective population sizes (N_e) of *Q. robur* and its dominant symbiotic partner *C. geophilum*.

Conclusion: Identifying common selective pressures acting on both, tree and fungal populations will help clarify co-adaptation processes, despite the challenge of detecting signals of local adaptation due to symbiotic interactions. Understanding the impact of extreme climatic events on both partners of a symbiotic mycorrhizal relationship is essential for assessing processes of environmental adaptation and the underlying gene variants involved in natural selection.



Is bird egg sterile? Challenges of metabarcoding in a low-biomass microbiome study

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POSTER SESSION 2 - P02.149, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

How and when the first bacteria colonise the gastrointestinal tract (GIT) in vertebrates remains a key, yet difficult-to-test question in evolutionary biology. Several influential studies over the last decade have suggested that bacterial colonisation may begin during early embryonic development. While recent mammalian studies challenge the idea of in utero colonisation—pointing instead to birth as the starting point—the timing and mechanisms of gut colonisation in birds remain elusive, largely due to inadequate methodology for microbiota profiling of low-biomass samples, which results in contamination and other artefacts not being filtered out and incorrectly considered as symbiotic microbiota.

Are bird eggs initially sterile, or do females seed them with bacteria to influence early microbiome development? This is an important question, as early bacterial colonisers may shape the GIT and the immune system development, affecting survival and microbiome communities after hatching. Bird eggs are well protected by various mechanical and chemical barriers, including a cocktail of antimicrobial peptides, yet some pathogens (such as *Salmonella*) can still overcome the defences. Three mutually non-exclusive hypotheses can explain avian GIT microbiota recruitment: (1) Eggs are sterile at laying (Sterile egg hypothesis) and (2) colonizations occur after oviposition by horizontal trans-shell migration, and/ or (3) vertical transmission occurs from the maternal oviduct.

To elucidate the mechanisms contributing to the formation of the gut microbiota in avian embryo, we examined the microbial communities in freshly laid eggs (day 0; E0), developing embryos (E13) and the digestive tract of a wild passerine, the great tit (*Parus major*) in our recent study (Tesicky et al. FEMS Microb.Ecol. 2024). Employing a methodologically advanced approach combining 16S rRNA gene

metabarcoding and microbe-specific qPCRs, our protocol allowed us to reliably distinguish symbiotic bacteria from environmental contaminating and cross-contaminating bacteria.

Bacterial profiles in eggs and embryos were dominated by environmental contaminants. After filtering these out, we revealed that the avian egg is nearly sterile but acquires a slightly richer microbiome during embryonic development. Of the three potentially pathogenic bacteria targeted by the more sensitive qPCR, only *Dietzia*, was found in the E0 eggs (yet also in negative controls), in the E13 guts and in the female samples, which could indicate possible vertical transmission.

These findings demonstrate that in passerines the initial gut microbiota is dominantly established after hatching, aligning with dynamic microbiome shifts observed in nestlings. They also align with recent publications highlighting methodological shortcomings, particularly insufficient contamination control in previous studies as environmental contamination and cross-contamination are common yet often overlooked issues in many studies based on amplicon sequencing, especially those using low-biomass DNA samples. We will also discuss methodological strategies to minimise these issues. While broader taxonomic sampling is still needed, our findings suggest that despite 310 million years of divergence, birds and mammals may share similar microbiota colonisation strategies.

Understanding the mechanisms of early GIT microbiota establishment can shed light on parental effects in birds and contribute to basic knowledge of host-microbe evolutionary ecology as well as zoohygienic and veterinary measures that minimise the risk of disease transmission.



Micromanaging: The architecture and evolution of host control in a microbial symbiosis

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POSTER SESSION 2 - P02.130, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Endosymbiosis is foundational to the origin of complex life and plays a central role in both natural and manmade ecosystems. By enabling functional compartmentalisation and evolutionary innovation, symbiosis underpins ecological processes and services at multiple levels. However, the molecular mechanisms that govern the evolution and stability of endosymbiotic relationships remain poorly understood, particularly in nascent or facultative symbioses where the fitness optima of host and symbiont may differ considerably. As endosymbiont cells live inside their hosts, the hosts evolve to regulate and control the growth and metabolism of its symbionts, but experimental data testing what happens when disrupting these controls is lacking.

Here, we investigate host control in the ciliate–algae symbiosis between *Paramecium bursaria* and *Microactinium conductrix*, a uniquely tractable model of facultative endosymbiosis. In this system, algal symbionts are individually housed in peri-algal vacuoles, where they exchange photosynthetically derived carbon and oxygen for nitrogen, carbon dioxide, ions, and protection against grazers and viruses. Hosts dynamically regulate their symbiont load in response to environmental conditions, particularly light, balancing the cost of provisioning nitrogen with the benefit of receiving carbon. Previous work has identified two putative host control mechanisms: a “carrot”, in which nitrogen provisioning via the arginine-to-polyamine pathway promotes symbiont growth and sugar release; and a “stick”, in which digestion of symbionts enables the host to reduce algal load, with sensing of breakdown products via the glycan processing pathway signalling successful digestion.

To test how these control pathways contribute to symbiosis stability and host fitness, we chemically disrupted each control mechanism by supplementing specific pathway intermediates and monitored population-level growth of hosts and symbionts using flow cytometry across replicated cultures. Preliminary results show targeting these pathways chemically has the potential to shift the cost:benefit balance, and even disrupt the symbiosis and lead to population extinction. Furthermore, the response depends on the step in the pathway targeted, and in the case of the arginine-to-polyamine pathway, N supplementation outwith the pathway does not show the same effect. We are currently testing how these disruptions interact with light environment, a key ecological axis along which host and symbiont fitness optima diverge. By the time of the conference, we will also have completed fitness assays under light × control mechanism compound combinations, along with untargeted metabolomics to assess the broader phenotypic impact of control disruption.

Although layered control architectures have been identified in other symbioses (including coral–algae and legume–rhizobia systems), the integration of multiple host control mechanisms and their impact on phenotypic plasticity and symbiotic stability have not been experimentally tested. This work will provide novel insight into how host control systems are structured, and how they determine the robustness of symbiosis under environmental variability. These questions are increasingly urgent as climate change places critical symbioses – such as those in coral reefs and agricultural systems – under unprecedented stress.



Mitochondrial adaptation regarding different chloroplast retention capabilities in Sacoglossa

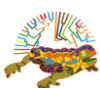
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POSTER SESSION 2 - P02.154, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

‘Solar-powered’ sea slugs belong to a small group called Sacoglossa within Heterobranchia and have the ability to sequester chloroplasts from its food algae. These ‘stolen’ plastids (known as kleptoplasts) are then stored in a still functional state in the slugs’ digestive gland cells. During this time the kleptoplasts continue photosynthesis inside the slugs building up a starch reservoir. The chloroplast retention abilities of sacoglossan species varies, and we distinguish between 1) non-function retention forms, in which the chloroplast does not stay active inside the slugs, 2) short-term retention forms, in which the chloroplasts stay active for a few weeks, 3) long-term retention forms, in which the chloroplasts stay active for several months, and 4) some forms, which do not incorporate the chloroplasts at all. Studies on plants

have shown that plastid and mitochondrial functions are interconnected to support essential cellular processes, such as energy production, metabolism and stress responses. However, the potential impact of kleptoplasts on mitochondria in sacoglossan species with varying chloroplast retention capabilities remains unexplored. Our aim is to search for mitochondrial adaptations/mutations in relation to their different chloroplast retention capabilities. We have assembled and annotated the mitochondrial genomes of 40 sacoglossan species representing all the different chloroplast retention forms. Phylogenetic analyses using IQ-TREE confirmed the most recent Sacoglossa phylogeny. We will analyze potential selective pressure on individual mitochondrial genes, using the programs PAML and HyPhy.



Pathogen Discovery in Ancient Cattle Remains: Possible Cause of Taurine Extinction in Thailand

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POSTER SESSION 2 - P02.118, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Cattle were domesticated in Thailand for thousands years ago and genetic evidence of cattle remains dated 1700-3500 years before present (YBP) indicated that *Bos taurus* or taurine were domesticated in Central and Northeastern Thailand during the prehistoric period. However, nowadays *Bos indicus* or zebu cattle are only traditional cattle in this country. The findings suggested that the extinction of Thai taurine had occurred. In the past, the importation of zebu into Africa caused a massive reduction of taurine (90%) due to an infectious disease but the zebu which had higher resistant to tropical diseases was killed only 30%. Hence, it was possible that the extinction of taurine in Thailand was due to an epidemic of infectious diseases after zebu had imported into the country. In this study, we aimed to identify cattle species and reveal pathogenic agents in the ancient cattle remain in Thailand in the Dvaravati period in which Indian culture had influence in the country.

The cattle remain was identified by DNA and morphology. Next Generation sequencing was performed and then genomic data were used to identify cattle species and classify pathogens. The results showed that the cattle remain belonged to taurine or a hybrid between taurine and zebu. The finding suggested that zebu may have been introduced into Thailand in the Dvaravati period. The total of 26 pathogenic bacterial species and 5 pathogenic viruses causing death in cattle and other mammals were detected. Some of them can kill cattle and humans including *Yersinia* sp. which had killed millions of humans in the past. *Campylobacter* sp. detected in this study can lead to difficult pregnancies and abortion in cattle and humans in present day. The result contributed that the pathogens causing death in cattle detected in this study have circulated in the cattle population since the Dvaravati period and some of them may cause the extinction of taurine in Thailand.



Patterns of correlation between mycorrhizal specificity and host genomics in *Platanthera* orchids across Eastern North America

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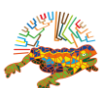
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POSTER SESSION 2 - P02.155, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Orchids, with their obligate dependence on specific mycorrhizal fungi and often narrow ecological niches, provide a powerful system for investigating how microbiome associations influence plant ecology and evolution. All orchids require compatible orchid mycorrhizal fungi (OMF) from their environment to germinate and establish seedlings, making fungal availability a key determinant of their local distribution. Most orchid species associate with a limited range of fungal partners, and it has been hypothesized that specialization to particular fungal lineages may contribute to lineage differentiation and species diversification in orchids.

To explore this, we identified OMF partners in 19 taxa in the terrestrial orchid genus *Platanthera*, from populations across

Eastern North America from Wisconsin and New Hampshire to Florida. We integrated fungal association data with host population genomic analyses to assess (a) whether OMF associations and degree of specialization differs among related orchid species and (b) whether more closely related orchid species have more similar OMF than distantly related orchid species. We found that most orchid species consistently associated with distinct clades of OMF, but that level of specificity varied. Orchid species which are known to form natural hybrids, exhibited broadly overlapping fungal associations. Our data indicate a strong correlation between host genomics and OMF partners. We discuss how host-microbiome interactions may contribute to speciation and diversification in orchids.



Persistence of an Influential Passenger: The Endosymbiotic Association of *Wolbachia* with the Neotropical Fly *Drosophila willistoni*

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POSTER SESSION 2 - **P02.150**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

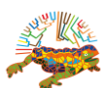
Background: The endosymbiotic bacterium *Wolbachia* is widespread in arthropods, largely due to its remarkable capacity to manipulate host reproduction and confer fitness-related traits. These traits—such as antiviral protection, altered host behavior, and increased fecundity—are often modulated by environmental factors, especially temperature. Temperature affects *Wolbachia* bacterial densities (titer), maternal transmission efficiency, and the expression of symbiont-conferred phenotypes, making it a key environmental variable in host-symbiont dynamics. While high *Wolbachia* titers are often necessary for beneficial traits like pathogen protection, they may also impose costs to the host. Despite extensive research in model organisms, little is known about how these dynamics play out in non-model Neotropical species, where *Wolbachia* presence and diversity remains underexplored.

In *Drosophila willistoni*, a widely distributed Neotropical species, *Wolbachia* prevalence is variable and driven by factors that are not yet well understood. Previous work suggests the presence of two infection types: one systemic, where *Wolbachia* is present in every host cell; and, one restrict, where *Wolbachia* is present only in neural cells and in the germline. Moreover, unlike other hosts, there is little evidence of *Wolbachia* manipulating reproduction in *D. willistoni*, raising the question of what evolutionary forces sustain these infections. Here we aim to: (1) assess whether *Wolbachia* titer is stably inherited across different temperatures; (2) determine if variations in titer are associated with distinct bacterial strains; and (3) evaluate how titer levels and strain identity correlate with host fitness and antiviral protection. By investigating these aspects, we seek to test known assumptions and enhance our understanding of host-symbiont interactions in diverse ecological contexts.

Materials and Methods: We collected 80 isofemale lines of *D. willistoni* from the Atlantic rainforest. We assessed *Wolbachia* infection using PCR and qPCR in whole females and dissected ovaries. Titer levels were quantified across different rearing temperatures (18°C and 25°C), ages, and tissues. Selected lines were subjected to reciprocal crosses with uninfected counterparts (UN) to evaluate reproductive compatibility. To test for antiviral protection, flies were challenged with *Drosophila C virus* (DCV). Representative high-titer (HT) and low-titer (LT) strains were genome sequenced.

Results: *Wolbachia* was detected in 61.3% of the isofemale lines. Based on titer, we classified 44 lines as high-titer (HT), 4 as low-titer (LT), and 31 as uninfected (UN). Infection prevalence was consistent with previous data from 2019, suggesting stable maintenance. HT strains showed systemic infection across tissues and maintained stable titers across temperatures and fly ages. LT strains were unstable and were lost in the laboratory over the generations. Crosses involving HT and LT lines and *D. paulistorum* revealed partial reproductive isolation and asymmetric maternal transmission. Genomic analysis showed the HT strain closely related to wWil and wAu, while the LT strain resembled wMel. Only the LT strain carried CI-associated genes. Despite high titers and systemic distribution, no antiviral protection against DCV was observed in HT-infected *D. willistoni*.

Conclusions: Our results reveal the coexistence of two divergent *Wolbachia* strains in a single *D. willistoni* population, each with distinct genomic signatures and infection dynamics. The HT strain appears to be stable, while the LT strain likely represents a recent horizontal acquisition. The absence of reproductive manipulation and antiviral protection, in systemically infected flies, suggests that its maintenance may rely on other ecological or evolutionary mechanisms. This study underscores the importance of examining the diversity of strategies used by endosymbionts to persist.



Phylosymbiosis and other factors shaping gastrointestinal microbiota composition of captive parrots: interspecific and intraspecific variation revealed by large-scale profiling

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POSTER SESSION 2 - **P02.134**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Phylosymbiosis, patterns of host-associated microbial community structure driven by evolutionary history of host species, is most often studied in various invertebrates or mammals, but only scarcely in birds. Captive parrots (Psittaciformes) combine the advantages of laboratory models (uniform environmental conditions) and wild animals (high diversity of species available), which makes them convenient models for host-microbiota association studies. Furthermore, domestic parrots are susceptible to numerous health issues that can be linked to gastrointestinal tract microbiota (GM) either directly or via gut-brain axis, highlighting the veterinary relevance of GM-oriented research in this group of hosts. Yet, the parrot GM is highly underexplored. Here, we addressed the interspecific and intraspecific variation in healthy parrot-associated GM, questioned the level of phylosymbiosis and identified the main factors shaping GM composition and diversity. We analysed faecal and oral microbiota of 277 individuals from 66 parrot

species through 16S rRNA gene profiling. Faecal and oral microbiota differed in composition, with oral microbiota having significantly higher alpha-diversity and lower interindividual variation. We did not find any core OTU or bacterial genus that would be present in faecal or oral samples of all individuals or species studied. We revealed high interspecific and intraspecific variation in both faecal and oral microbiota, with host species and owner identity being the most important factors influencing microbial composition and diversity, followed by sex and season. Individuals representing the same parrot species or genus shared similar GM communities, but no clear phylogenetic signal was observed in GM composition among more distantly related host taxa. These results indicate that the parrot-GM reciprocal adaptations lead to phylosymbiosis across closely related lineages, but the signatures of these interactions fade at higher phylogenetic levels.



Plant conservation hologenomics: combining population genomics with microbiont community assessments for two orchid species in Denmark

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POSTER SESSION 2 - P02.121, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

As the importance of the microbiome for plant health and ecology becomes increasingly evident, it seems clear that effective conservation of wild plant species needs to integrate knowledge on both the plants and their microbionts, i.e., the holobiont. Conservation of the genetic diversity in a plant species could, therefore, be extended to involve considerations for its diversity of functionally important microbionts. Population genomics offers valuable tools for designing better conservation strategies for vulnerable plant species, but does not take into account host-specific interactions with and dependence on specific microbionts. We propose that integrating population genomics with host microbiome studies into a wider plant conservation hologenomics context is needed for the conservation of the plant holobiont. This approach offers novel levels of understanding habitat requirements,

landscape genomics and identification of evolutionary significant units. With their dependence on specific mycorrhizal fungi (OMF), orchids provide a great illustration of the importance of the microbiome component in conservation studies. While it is known that most orchids have specific requirements for their mycorrhizal partners and that abundance of OMF in the soil effects distribution of the orchids, there is a scarcity of knowledge on how the orchid mycobiome varies with populations and across the landscape, and how it correlates with host genomic variation. We here demonstrate an orchid conservation hologenomics approach by combining population genomics with root mycobiome data for two closely related orchid species (*Platanthera chlorantha* and *P. bifolia*) across populations in Denmark, and discuss the implications of our findings for conservation efforts.



Quantifying the impact of insect host genotype on Wolbachia titer

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¹Cbgp Inrae

POSTER SESSION 2 - **P02.141**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Wolbachia are the most abundant endosymbiotic bacteria found in arthropod species, known to induce a wide variety of phenotypes on their hosts, with strong evidence indicating that some fitness-associated host traits depend on bacterial load.

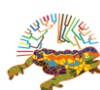
Here we quantify how Wolbachia titer is influenced by the genome of its host, in *Drosophila melanogaster*, using the *Drosophila* Genetic Reference Panel (DGRP).

We first found a large variation in Wolbachia titers among 90 DGRP lines, using read mapping coverage data to estimate titers in silico.

We found high broad-sense heritability for Wolbachia titer variation. To identify the host loci associated with this phenotype, we performed a genome-wide association study using 1,202,368 biallelic SNPs.

Finally, we validated our estimation of Wolbachia titers in silico using direct estimation with qPCR.

We discuss our results in light of previous studies in *D. melanogaster* and *D. simulans*, and we conclude that our research brings further evidence of Wolbachia titer regulation by the host genome, illustrating the complexity of host-endosymbiont relationships.



Ruling the kingdoms: coordinated stress responses in a lichen-forming fungus partnered with algal or cyanobacterial symbionts

Goss A¹, Werth S¹

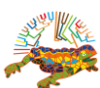
¹Ludwig Maximilian University of Munich

POSTER SESSION 2 - **P02.129**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Symbiotic associations play a central role in shaping the ecological niches and evolutionary potential of both hosts and their microbial partners. In lichens, photobiont identity can influence habitat range, physiology, and even reproductive strategy. Yet the contributions of each partner to environmental adaptation and specialization remain poorly understood.

The foliose lichen *Sticta canariensis* forms a rare photosymbiodeme composed of two morphotypes: one associating with the cyanobacterium *Nostoc* (cyanomorph), and the other with the green alga *Symbiochloris* (chloromorph). These morphs differ not only in symbiotic composition but also in phenotype and microhabitat preference, offering a valuable example of symbiont-driven ecological diversification.

To investigate the molecular basis of stress response in this complex symbiosis, we examined transcriptional responses to short-term temperature variation across the eukaryotic symbionts of *S. canariensis*. Using RNA-seq and co-expression network analysis, we observed morph- and temperature-specific transcriptional shifts. These included coordinated shifts in stress responses and metabolic processes. The observed coordination of stress responses reflects a key facet of phenotypic plasticity in the lichen symbiosis and may contribute to ecological specialization and long-term adaptability in the face of environmental change.



Social transmission of the microbiome in wild baboons

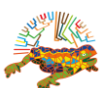
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POSTER SESSION 2 - P02.119, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Animals form stable associations with microbial communities that aid them in breaking down certain nutrients and in developing their immune systems. However, many gut bacteria have evolved to become so specialized that they can no longer survive outside of their hosts, raising the question of how animals acquire members of their microbiome that they are unlikely to encounter in their environments. One theory is that close social interactions promote microbial transmission, allowing animals to pass around specialized bacteria without losing them, and that selection on microbiome transmission may therefore contribute to the evolution of some forms of social behavior. To test this hypothesis, we integrated strain-resolved metagenomics with detailed behavioral and demographic data from the intensively studied population of wild baboons in the Amboseli ecosystem of Kenya. To characterize the gut microbiome, we collected fecal samples from a population of 132 baboons—all of the adult members of five social groups (n=398 samples). Transmission occurred more often

between members of the same social group than between members of different social groups. Within social groups, transmission was most common among close social partners that frequently engaged in grooming behaviors. Baboons that had recently migrated between social groups were disproportionately involved in transmission events, suggesting that dispersing animals bring new strains or species that their group members may not have encountered. Finally, bacterial species whose transmission patterns followed the social network remained in their hosts for significantly longer durations than bacterial species whose transmission patterns did not correlate with the social network, suggesting that bacteria that rely on social transmission are more likely to be particularly adapted to their hosts. Our results suggest that social behaviors can facilitate microbial transmission, particularly of specialized bacteria, and may provide insight into how animal-microbiome associations and animal social behaviors evolved.



Symbiopectobacterium in Rhodnius prolixus: evidence for a transitional intracellular symbiosis

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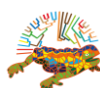
POSTER SESSION 2 - **P02.127**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

It is well established that obligate blood-feeding insects often rely on symbiotic associations with bacteria to supplement essential nutrients, particularly B vitamins, which are deficient in a blood-only diet. Most of these insects harbor obligate symbionts housed within specialized cells or organs known as bacteriomes. Interestingly, kissing bugs (Hemiptera: Reduviidae) appear to diverge from this pattern by maintaining extracellular associations with bacteria localized in the gut lumen rather than in bacteriocytes. Among these potential symbionts, *Symbiopectobacterium* has previously been identified as a symbiont of *Rhodnius prolixus*. This bacterium has also been detected in diverse hosts, including nematodes, fruit flies, leafhoppers, and bullrush bugs, where in the latter it functions as an obligate symbiont residing within bacteriomes.

To investigate the localization and the symbiotic role of *Symbiopectobacterium* in *R. prolixus*, we prepared histological sections from the whole length of the gut (including crop and rectal ampula) dissected from later developmental instars (L4,L5) and adults for fluorescence in situ hybridization (FISH). Probes specific to *Symbiopectobacterium* as well as general eubacterial 16S rRNA probes were used to detect bacterial presence and localization. FISH analysis revealed that *Symbiopectobacterium* is localized intracellularly within the epithelial cells of the posterior midgut. No evidence of

intracellular colonization was observed in the crop or rectal ampula. Unlike the dense and uniform colonization typically observed in specialized bacteriomes, the distribution within the midgut cells of *R. prolixus* is uneven. Not all gut epithelial cells were colonized, and the number of bacteria varied significantly between cells. Most bacteria appeared to accumulate near the gut lumen, although in some cells, bacteria were dispersed throughout the cytoplasm. This intracellular localization sets *Symbiopectobacterium* apart from other facultative symbionts of many other hemipterans, such as stinkbugs, which house beneficial bacteria extracellularly in midgut crypts. Supporting its potential nutritional symbiotic role, genomic analysis of *Symbiopectobacterium* recovered from the *R. prolixus* colony showed complete biosynthetic pathways for multiple B vitamins, including thiamine (B1), riboflavin (B2), pantothenate (B5), pyridoxal (B6), biotin (B7), and folate (B9). Additionally, the genome encodes a functional type III secretion system, which may be important in host cell invasion and intracellular persistence.

These findings suggest that, unlike previously assumed, *R. prolixus* enters a more intimate interaction with *Symbiopectobacterium*. The bacteria are intracellularly localized within the epithelial cells of the posterior midgut, likely representing a transitional form of symbiosis.



Temperature mediates coevolution between parasitoids and symbiont-protected hosts

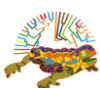
Vorburger C^{1,2}, Stalder D^{1,2}, Gimmi E^{1,2}, Wallisch J¹, Hudson C¹

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POSTER SESSION 2 - **P02.126**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Heritable bacterial symbionts often evolve the ability to defend hosts against parasites. Parasites exploiting hosts protected by defensive symbionts are therefore faced with two lines of defense: Those encoded by the host and those conferred by the symbiont. Black bean aphids (*Aphis fabae*) show genotypic variation in resistance to an important natural enemy, the parasitoid wasp *Lysiphlebus fabarum*. Part of the aphid population is additionally protected by infection with the heritable bacterial endosymbiont *Hamiltonella defensa*, which increases resistance to *L. fabarum*. In comparison to the host-encoded resistance, the resistance conferred by *H. defensa* is both stronger and more specific, in that different symbiont strains are unequally effective against different parasitoid genotypes. Experiments and models have shown that in this situation, the symbiont can 'take over' the coevolution with the parasite. However, defensive symbionts tend to have multiple effects on their hosts, of which protection against parasites is just one, raising the possibility that other environmental factors can interfere with the selection by parasitoids. We have learnt – initially by accident – that in black bean aphids, this applies to temperature. In the field, we observed that seasonal increases in

H. defensa prevalence are better predicted by periods of high temperature than by parasitism risk, and in the lab, we observed occasional losses of *H. defensa* in aphid lines reared at low temperature. This suggests that *H. defensa* may protect against heat stress and that vertical transmission of this symbiont is less reliable at low temperatures. Both effects could be confirmed by targeted experiments. This yields the prediction that infections with *H. defensa* should be more prevalent in warmer areas of the host's geographic distribution. We tested this prediction by sampling black bean aphids along a transect from southern Italy to northern Sweden. Indeed, there is a strong latitudinal cline in the prevalence of *H. defensa*, declining from approx. 80% prevalence in the southernmost sites to 0% infection in the northernmost sites. Temperature thus appears to be driving a cline in symbiont-conferred resistance in European black bean aphids. We predict that this has important consequences for coevolution with *L. fabarum*. The relative importance of symbiont-conferred vs. host-encoded resistance will shift along this cline, requiring different counteradaptations by parasitoids in southern vs. northern populations.



The influence of gut microbes on the social brain

Liberti J¹

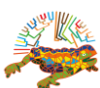
¹University Of Geneva

POSTER SESSION 2 - P02.131, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The gut microbiota is increasingly recognized for its influence on brain function, with consequences for social behavior. Most studies have focused on the clinical perspectives of modulating the microbiota-gut-brain (MGB) axis for the treatment of neurodegenerative diseases and behavioral dysfunctions, using experimental models of human genetics and disease. However, it is still unclear to what extent the MGB axis modulates social interactions across the tree of life and whether the proximate mechanisms regulating these microbiota-host interactions are conserved. Social bees provide unique models to advance our understanding of the MGB axis and its role in shaping social interactions, as they exhibit complex social behaviors at both the individual and collective levels.

To study the effects of bacterial symbionts on brain physiology and social behaviors, we manipulated the composition of the honeybee

gut microbiota and coupled these manipulations with automated behavioral tracking. We discovered that the gut microbiota increases the frequency and specialization of social interactions among bees, and that these effects are associated with changes in gene expression and an increase in the concentration of specific metabolites in the bee brain. We are currently performing follow-up studies to disentangle the relative contributions of individual gut members to distinct social behaviors and further unravel the molecular mechanisms mediating these interactions. Additionally, we are exploring the evolutionary conservation of microbiota – social brain interactions across bee lineages and assessing their ecological significance. Our results suggest that the gut microbiota plays an important role in shaping the social organization of bees, with potential consequences for their ecological success and the services they provide as pollinators.



The Living Fossils of Symbiosis: Wolbachia Genes in *Rhodnius prolixus*

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POSTER SESSION 2 - **P02.140**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The advent of high-quality arthropod genome assemblies enables the discovery of evolutionary relics from past symbiotic relationships. A near-chromosome-level assembly of the *Rhodnius prolixus* genome (in prep.) reveals 129 horizontally transferred genes or gene fragments of Wolbachia origin; substantially more than the 27 genes across 85 regions reported in the earlier assembly (GCA_000181055). Our results highlight the compact nature of nuclear Wolbachia transfers, with 107 genes clustered within a single 176 Kb region. Phylogenetic analysis indicates their origin from the Wolbachia F supergroup. Unlike in other hosts (e.g., bean beetle, pine sawyer beetle, fruit fly, or tsetse fly), where Wolbachia-derived regions progressively decay, over 50% of these genes are actively transcribed in *R. prolixus*, often in a tissue-specific manner, with strong

enrichment in ovarian expression. The most represented functional categories among transcribed genes include cell wall/membrane/envelope biogenesis (COG category M, n=15) and replication, recombination, and repair (COG category L, n=11). The presence and expression of genes encoding transposases, DNA polymerase I, reverse transcriptase, helicases, and mismatch repair proteins (20 total; 11 expressed) suggest a potentially functional Wolbachia-derived machinery for genome plasticity and maintenance. Codon usage patterns closely match host preferences, implying co-option of Wolbachia genes by the *Rhodnius* genome. Functional validation through gene knockdowns is however essential to assess their roles in host-microbe interactions.



The microbiome of suprapubic and sternal scent glands of wild tamarins suggests microbes play a key role in olfactory communication

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POSTER SESSION 2 - **P02.122**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The microbiome of mammalian scent glands is increasingly realised to play a key role in olfactory communication. Defining these glandular microbiomes in wild species is essential for understanding the complex evolution of host-microbe interactions and their influence on scent production. We studied the microbiome of suprapubic and sternal scent glands in two sympatric species of wild primates in Peru: saddleback tamarins (*Leontocebus weddelli*), and emperor tamarin (*Saguinus imperator*). Sternal and suprapubic glands have similar histology, but the suprapubic gland is used more frequently for scent marking behaviors, possibly favouring more microbial exchange through environmental contact. We aimed to: 1) profile sternal and suprapubic glandular microbiomes of both primate species, focusing on microbial taxa and metabolic pathways related to odor production, and 2) determine if suprapubic glands had higher diversity and distinct composition of odor-related taxa and pathways compared to the sternal glands.

We collected 39 swabs of suprapubic glands (19 saddleback, 20 emperor) and 37 swabs of sternal glands (18 saddleback, 19 emperor). We generated metagenomic libraries and used short-read DNA shotgun sequencing on an Illumina NovaSeqX. We profiled the glandular microbiome of the two tamarin species by calculating relative abundances and prevalence of microbial taxa, and we assessed differences in microbial diversity and composition between gland types. We used GLMMs to analyze alpha-diversity (Shannon and Chao1 indices), PERMANOVA to analyze beta-diversity (robust Aitchinson index), and ANCOM-BC tests to

examine differences in abundances of taxa and pathways.

The microbial profile of our entire datasets, comprising suprapubic and sternal glands of both *L. weddelli* and *S. imperator*, included 74.5% of bacterial taxa and 25% of eukaryotes. We identified the same 18 odor-associated microbial taxa in each tamarin species, mainly in genera *Staphylococcus* and *Corynebacterium*, and 26 shared pathways, such as pyruvate fermentation and amino acid metabolism. Suprapubic glands differed in beta-diversity and had lower Shannon alpha-diversity relative to sternal glands, and the effect was more pronounced in *L. weddelli*. The glands of *L. weddelli* also differed in taxonomic composition, with odor-related taxa such as *Corynebacterium atypicum* and *Corynebacterium frankenforstense*, more abundant in suprapubic glands.

Our results provide new insight into the taxonomy and the metabolic activity of the glandular microbiome, reinforcing hypotheses of their key role in odor production. The significant differences in microbial diversity and composition between sternal and suprapubic glands of *L. weddelli* suggest that the microbiome of suprapubic glands play a more specialized role in odor production than the sternal glands. This is in contrast to the glands of *S. imperator*, highlighting potential species-specific variations in glandular microbiome adaptations.

This study offers a novel perspective into the role of microbiomes in shaping the odor of wild animals, uncovering microbial taxa and pathways similar to those found in humans and other mammals, and highlighting putative microbial adaptations for olfactory communication.



The rat gut microbiome is affected by indirect genetic effects from social partners, which could drive rapid evolutionary change

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POSTER SESSION 2 - **P02.137**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Mammalian hosts have evolved mechanisms to actively filter and select the symbiotic microbes that live inside their gut. This ongoing evolutionary process has left signatures in the form of associations between host genotypes and gut microbiome traits. In addition, mammals exchange gut microbes through vertical and horizontal transmission. This ecological process can now be observed directly by characterising the microbiome at the level of bacterial strains and tracking strain transmission across hosts.

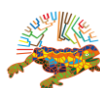
We hypothesised that the coupling of these evolutionary and ecological processes would result in indirect genetic effects on the microbiome (Mic-IGE), whereby microbiome traits of one individual are affected by genotypes of other, conspecific individuals (social partners). Based on established evolutionary theory on indirect genetic effects, we further hypothesised that Mic-IGE would increase the total genetic variance of microbiome traits, which would greatly increase the potential for evolution by natural selection.

To test these hypotheses, we studied host/microbiome interactions in outbred “Heterogeneous Stock” (HS) laboratory rats

housed in groups of two or three. HS rats show high levels of genetic diversity and heterozygosity, and the genetic variants segregating in the HS are natural, inherited from the wild ancestors of the population. In addition, like their wild conspecifics, HS rats have a complex gut microbiome and they express social behaviours (e.g. allo-coprophy) favouring gut microbial transmission between social partners.

We sequenced and profiled the gut (cecal and fecal) microbiomes of thousands of HS rats using various, complementary sequencing techniques. The genotypes of the rats were available from collaborators. We carried out an in-depth analysis of direct and indirect genetic effects on the microbiome, focusing on Mic-IGE arising between adolescent and adult rats sharing a cage.

We found pioneering evidence of Mic-IGE in this experimental setting. We further demonstrated that, as expected, Mic-IGE greatly increased the total genetic variance. We integrated these empirical results with state-of-the-art evolutionary theory on indirect genetic effects to conclude that Mic-IGE could drive rapid evolutionary change in host/microbiome interactions.



Tissue Type drives plant-associated bacterial community structure more than geographic location: Insights from Antarctica and Patagonia

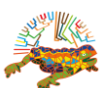
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POSTER SESSION 2 - P02.151, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Plant-associated bacterial communities are crucial in understanding evolutionary adaptations of plants to the environment and changing climatic conditions. Plant recruit bacteria that contribute nutrition, disease resistance and stress tolerance based on biotic and abiotic conditions, ensuring the host plant survival. Plant bacterial communities are often described from tropical and temperate environments. However, bacteria associated with plants in polar regions remain elusive, especially from southern polar regions. This is particularly intriguing since plant-bacterial adaptations in vascular plant *Deschampsia antarctica* in Antarctica gives insights into the evolutionary adaptations of primary successors in an ecological community. With changing climatic conditions, Antarctica is being warmer and there can be expansion of range of species. The present study accounts for the bacterial communities in *Deschampsia* in Patagonia and Antarctica and understands their importance in these extreme environments. Leaf, root and phyllosphere samples were collected from 4 different populations in

Antarctica and 6 different populations in Patagonia. DNA was extracted from each tissue sample and gene libraries were prepared by amplifying V6-V8 regions of the 16S rRNA gene. Using Illumina MiSeq platform, the 16rRNA gene sequencing was done to find bacterial communities. Using phyloseq, DADA2 and microbiome packages in R, data analyses was performed. In both locations, root endophytic community and leaf endophytic community were diverse than phyllosphere communities. But remarkably, we found that the bacterial communities are specific to tissue type rather than geographical locations. This strongly shows the evolutionary relations between plant tissue niche and the bacterial communities. In addition, certain bacterial taxa were found to be specific to Antarctica and Patagonia. This could indicate their special significance related to niche, geographical location or climatic condition. This needs to be further investigated to understand their functional relevance based on geographical location and evolutionary significance.



A genome-eroded symbiont with a transcriptional response to the host's needs

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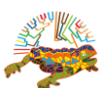
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MEETING ROOM 116, August 21, 2025, 10:30 AM - 12:30 PM

Symbiotic bacteria in long-term host associations frequently undergo extreme genome reduction. While they retain genes beneficial to the host, their repertoire of transcription factors is severely reduced. Here, we investigate the gene expression of genome-eroded symbionts across life-stages and species of a beetle subfamily: the reed beetles (Coleoptera: Chrysomelidae, Donaciinae). These symbionts feature a small genome (~0.5 Mb), encoding for 9-10 essential amino acid biosynthesis pathways, 0-2 pectinases, and 4-5 transcription factors. Across life-stages, symbionts adjusted gene expression to meet the hosts' nutritional demands, upregulating amino acid (AA) biosynthesis in larvae, while expression and enzymatic activity of host and symbiont enzymes involved in plant cell-

wall break-down increased in the folivorous adults. Although the upregulated symbiont genes during larval stages are enriched for AA biosynthesis genes across all species, different AA pathways were upregulated across genera. These differences correspond in part to the presence/absence of different AA pathways in the symbiont's genomes and differences in the cocoon's amino acid composition, indicating a symbiont's contribution to cocoon building. Thus, reed beetle symbionts show a functionally consistent transcriptional response to host demands, while showcasing transcriptomic changes related to differential gene losses, highlighting the regulatory potential of long-term coevolved symbionts despite severely reduced genomes.



Could co-evolution explain Wolbachia-driven loss of pesticide resistance in spider mite populations?

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MEETING ROOM 116, August 20, 2025, 10:30 AM - 12:30 PM

Background: The ubiquity of Wolbachia in arthropods and its ability to manipulate host reproduction have attracted considerable attention for its use as a gene drive vehicle to control pest populations. Such control strategies are generally based on genes carried by Wolbachia itself, but their success also hinges on the potential effect of this symbiont on the dynamics of host genes, such as those responsible for adaption to particular environmental pressures, including other control strategies. We addressed this issue with a combination of theoretical and experimental approaches.

Methods and Results: We first showed theoretically that, given the range of fitness effects that Wolbachia can have on their hosts, even transient association between different host genotypes and this endosymbiont may lead to the fixation or the elimination of host alleles.

Then, we performed experimental evolution of spider mite populations sharing the same genetic background but carrying or not a single nucleotide mutation conferring resistance to the pesticide etoxazole, and infected or not with Wolbachia. We found that the host genotype alone (fully resistant vs. fully susceptible populations) did not affect the spread of Wolbachia, nor did Wolbachia infection alone (fully infected vs. fully uninfected populations) affect the dynamic of the resistance allele. However, we found variable dynamics of the resistant allele when Wolbachia was simultaneously invading the host populations: the frequency of the resistance allele remained constant when Wolbachia infection was associated

with susceptible hosts at the beginning of experimental evolution, whereas it dropped when the symbiont originated from resistant hosts.

Finally, we measured the effect of Wolbachia infection on the life history traits of different host genotypes (homozygous susceptible or resistant, or heterozygous), as well as the effect of the pesticide resistance allele on Wolbachia-induced cytoplasmic incompatibility (CI). Preliminary results show that, in compatible crosses, Wolbachia infection increases the growth rate only of hosts carrying the etoxazole resistance allele, and, in incompatible crosses, Wolbachia-infected resistant males induce lower CI levels than Wolbachia-infected susceptible males.

Conclusions and Perspectives: One possibility to explain such genotype-specific fitness effects of infection, and observed dynamics of the resistance allele, hinges upon the long co-evolutionary history between this allele and Wolbachia in the host populations. Even though Wolbachia infection was introgressed via repeated backcrosses from the susceptible populations into the resistant ones, ensuring the same strain initially infected all populations, this was done more than a hundred host generations before performing the experiments. Although these results call for further investigations to determine whether and how co-evolution would explain this outcome, they also undoubtedly show that Wolbachia can play an important role in the evolutionary trajectories of its hosts.



Dissecting the fitness components of nitrogen-fixing rhizobia throughout their symbiotic life cycle

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MEETING ROOM 116, August 21, 2025, 10:30 AM - 12:30 PM

Living organisms are surrounded by complex microbial communities, where interactions, ranging from mutualistic to pathogenic, influence their physiology, development, and ecological success. These associations span from transient encounters to intimate symbioses, shaping evolutionary trajectories on both sides. Among the most well-studied mutualistic interactions is the nitrogen-fixing symbiosis between legume plants and rhizobia. During this interaction, bacteria are hosted in specialized root organs called nodules, where they provide organic nitrogen to the host in exchange for carbon compounds. This mutualism enhances the fitness of both partners. From the rhizobial perspective, fitness comprises multiple components that are important at different life cycle stages, including: (i) proliferation and survival in the soil and rhizosphere, (ii) nodulation efficiency and competitiveness, (iii) proliferation and survival within nodules, and (iv) release from nodules during senescence. The role of host-related factors in shaping rhizobial fitness has been deeply studied. However, the contribution of interbacterial interactions to rhizobial fitness and evolutionary dynamics is still poorly understood.

In this study, we examined how interactions among nine beta-rhizobial strains affect their fitness during symbiosis with the legume plant *Mimosa pudica*. We first characterized the fitness of each strain in single-inoculation experiments, measuring

nodulation rate, proliferation within nodules, and nitrogen fixation. These traits were used to predict the outcomes of pairwise co-inoculations under the assumption that no interaction occurs between strains. We then performed 36 pairwise co-inoculation experiments to test these predictions. In most pairs, observed fitness differed from predictions, indicating that ecological interactions among rhizobial strains are common. Nodulation competitiveness, defined as the ability to form nodules during co-inoculation, was the primary factor modulating interactions among strains. This finding highlights the role of early infection steps as a bottleneck for rhizobial selection. However, we also identified a case where co-inoculation affected rhizobial proliferation within nodules, suggesting indirect, potentially host-mediated, interactions during later stages of symbiosis.

Additionally, we tested more complex communities composed of six or eight strains to assess the transitivity of competitive interactions. Fitness rankings established from pairwise experiments predicted outcomes in these multistrain communities, suggesting that, in this system, higher-order interactions are either negligible or have only a limited effect on rhizobial fitness.

Overall, this work provides a detailed account of the ecological processes governing symbiotic fitness in simple communities of plant-mutualistic bacteria.



Eco-evolutionary context shapes fitness and diversity in a protective symbiosis

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MEETING ROOM 116, August 20, 2025, 10:30 AM - 12:30 PM

Microbial symbionts protect many host species against pathogens. Ecological and coevolutionary context can shift rapidly in these networks of interacting species, and how such shifts affect the trajectories of each player remain important questions. When under attack from a coevolving pathogen, will hosts respond by depending on their symbionts, or by investing in their own defence mechanisms? In turn, if a pathogen becomes extinct in a community, how will the interaction between host and symbiont proceed? To address such

questions, we experimentally evolved a community of genetically-diverse nematode hosts, protective symbionts (*Enterococcus faecalis*) and pathogens (*Staphylococcus aureus*) under different eco-evo contexts. After 15 host generations we report effects on host dependence, pathogen virulence and symbiont diversity. Our results highlight inherent complexity in the evolution of protective symbioses and hold relevance for programs that introduce symbionts for pathogen control.



Evolutionary dynamics of nested symbiosis in *Aphrodes* leafhoppers

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MEETING ROOM 116, August 21, 2025, 10:30 AM - 12:30 PM

Background: Symbiotic associations with microbes have played a central role in insect diversification and ecological success. In Auchenorrhyncha – a group that includes leafhoppers, planthoppers, spittlebugs, and cicadas – these associations originated ~300 million years ago when their common ancestor acquired two bacterial symbionts: one from the phylum Bacteroidetes and another from the phylum Proteobacteria (class Betaproteobacteria). Descendants of these ancestral symbionts persist in many modern lineages, though some clades have since replaced or supplemented them with new partners, leading to remarkable diversity in symbiotic systems. Leafhoppers of the genus *Aphrodes* exhibit a particularly intriguing system that illustrates both long-term evolutionary conservatism and ongoing diversification of symbionts. In addition to the two ancient symbionts – *Sulcia* (Bacteroidetes) and *Nasuia* (Betaproteobacteria) – our research reveals a third partner, which is the gammaproteobacterial symbiont *Sodalis* localized within the cytoplasm of *Sulcia* cells. This represents a rare and highly unusual case of nested symbiosis, where one bacterial symbiont lives inside another.

Materials and Methods: To explore this unique nested symbiosis, we used light and electron microscopy to localize *Sodalis* in the *Aphrodes* body. To get a broad picture of microbial diversity, we generated 16S rRNA amplicon data for 112 individuals representing 3 *Aphrodes* species, collected across 16 populations from mainland and island sites in Europe. Additionally, we performed metagenomic sequencing on a subset of 30 individuals. Assembly and contig clustering were used to detect co-occurring *Sodalis*

strains, and phylogenomic analyses, based on conserved, informative genes, were conducted to explore their evolutionary relationships.

Results: Microscopy revealed that *Sodalis* is localized within *Sulcia* cells, both in specialized bacteriomes and during transovarial transmission, suggesting its essentiality for the host and advanced intracellular adaptation. Amplicon data revealed population-level variation in *Sodalis* presence, with multiple strains often co-occurring within individual hosts. Metagenomic analyses confirmed the presence of distinct *Sodalis* strains co-occurring in several hosts. The *Sodalis* phylogeny was inconsistent with the host tree, suggesting multiple acquisition events or ongoing strain replacement, possibly involving horizontal transmission. Meanwhile, *Sulcia* and *Nasuia* genomes remain stable and highly reduced, retaining complementary pathways for essential amino acid biosynthesis. In contrast, *Sodalis* retains a broader metabolic range, consistent with a more recent integration into the symbiotic system.

Conclusions: The persistence of multiple *Sodalis* strains within individual hosts, along with their unique nested arrangement inside *Sulcia*, highlights the evolutionary dynamics of the *Aphrodes* symbiotic system – one that merges ancient partnerships with ongoing microbial exchange. This system offers a valuable model for investigating nested symbiosis, co-residence of symbionts, and symbiont replacement within a stable host framework. Our work underscores the importance of strain-level resolution in symbiosis research and illustrates the flexibility of microbial partnerships in shaping insect adaptation and diversification.



Infection dynamics of endosymbionts that manipulate arthropod reproduction

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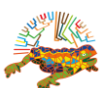
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MEETING ROOM 116, August 20, 2025, 10:30 AM - 12:30 PM

Endosymbionts capable of altering host reproduction are widespread among arthropods. These reproductive manipulations – including male-killing, feminisation, cytoplasmic incompatibility, parthenogenesis induction, and sex allocation distortion – have independently evolved across a diverse range of microorganisms, including bacteria, fungi, and some viruses. Whilst all these manipulations are adaptations of the symbiont to increase its spread within host populations, they differ in their mechanistic underpinnings and resulting infection dynamics.

Our study presents a unified modelling framework that captures the key elements required to describe the effect of each reproductive manipulation. This approach not only highlights fundamental insights from previous research on each manipulation but also enables direct comparisons of the infection dynamics resulting from different strategies. We derive invasion criteria for all possible combinations of manipulative endosymbionts – including previously unexplored scenarios – demonstrating the

conditions under which a rare manipulating endosymbiont can successfully invade a population that is either uninfected or already harbouring another reproductive manipulator. Additionally, we show when the spread of a reproductive manipulator can differ between haplodiploid and diplodiploid hosts, and when it does not, highlighting the importance of interactions between host and symbiont biology for endosymbiont spread. Intriguingly, our framework reveals that reproductive manipulations which, in principle, might be expected to have superior invasion prospects are not necessarily the most prevalent in nature. This suggests that successful spread of a reproductive manipulator is also strongly linked to the underlying physiological mechanisms of each manipulation and other aspects impacting interspecific host switches. These findings highlight the drivers of endosymbiont spread and persistence, which are essential to appreciating their evolutionary influence on arthropods and advance our understanding of host-symbiont coevolution.



Pushing the limits of genome reduction: evolution of ancestral planthopper symbiont genomes

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MEETING ROOM 116, August 21, 2025, 10:30 AM - 12:30 PM

Background: Ancient heritable nutritional endosymbiotic bacteria found in sap-sucking hemipterans are characterized by extremely reduced genomes and strict maternal transmission. These symbionts, essential for supplementing amino acids absent from the insect diet, have lost over 90% of their ancestral genes, resulting in some of the smallest bacterial genomes. After the intense genome reduction, symbionts reached a stable evolutionary stage in which their remaining genes, critical for the host-symbiont relationship, maintain highly conserved content, structure, and organization. However, in multiple host lineages, these tiny genomes undergo further dynamic changes, including further genome reduction, rearrangements, and fragmentation. In our study, we investigated these evolutionary processes in planthoppers (infraorder Fulgoromorpha), a diverse group of Auchenorrhyncha that are associated with two ancient, bacterial symbionts with highly reduced genomes: *Candidatus Sulcia muelleri* and *Candidatus Vidania fulgoroidea*, for approximately 263 million years.

Materials and methods: Metagenomic libraries from 149 globally collected planthopper species were sequenced using Illumina. *Sulcia* and *Vidania* genomic fragments were identified in Megahit metagenomic assemblies using BLAST tools and previously curated reference genomes. Annotations of these highly divergent genomes, especially of *Vidania* characterized by extreme sequence evolutionary rates, were done through iterative HMMER searches using collections of hand-curated gene alignments. Genome organization was reconstructed based on annotations and comparison with the reference *Sulcia* and *Vidania* genomes from *Oliarus filicicola* OLIH.

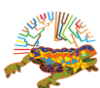
Results: Our results showed that *Sulcia* and *Vidania* are broadly distributed

across planthopper species. Based on rRNA sequences from metagenomic data, we identified *Vidania*, typically accompanied by *Sulcia*, in 87 out of 149 species, representing 15 of 19 surveyed families. We reconstructed 65 *Sulcia* and 68 *Vidania* circular genomes, with sizes ranging from 137–180 kb for *Sulcia* and 50–137 kb for *Vidania*. While *Vidania* genomes largely preserved ancestral gene order, approximately 21% of *Sulcia* genomes exhibited structural inversions.

Our comparative analyses revealed that the ancestral gene sets of *Sulcia* and *Vidania* symbionts comprised at least 164 and 169 genes, respectively. We detected no gene gains, but gene losses were common, particularly in *Vidania*, indicating ongoing genome erosion that primarily affects genes involved in genetic information processing and metabolism. The two smallest *Vidania* genomes from *Saccharosydne* sp. (50 kb) and *Malenia bosnica* (52 kb), exhibit striking convergence in gene content despite evolving independently, retaining core genes such as rRNA genes, ribosomal proteins, and polymerase subunits, while lacking all aminoacyl-tRNA synthetases and most amino acid biosynthesis pathways except for phenylalanine.

In some planthopper lineages, the ancestral *Sulcia* genome has split into multiple genetically and cytologically distinct but interdependent lineages that together function as a single symbiont.

Conclusions: Our study reveals the remarkable genomic diversity and evolutionary dynamics of the ancient symbionts *Sulcia* and *Vidania* in planthoppers. These findings enhance our understanding of the processes and drivers of genomic evolution of ancient symbionts and their co-evolution with host species.



Spread of Wolbachia in haplodiploids is driven by extensive rewiring of female reproductive physiology

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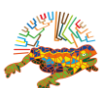
MEETING ROOM 116, August 20, 2025, 10:30 AM - 12:30 PM

Maternally inherited symbionts, such as Wolbachia, manipulate host reproduction to promote their transmission. Recent work has shown that Wolbachia can distort sex allocation in haplodiploid hosts by increasing egg fertilization rate, a reproductive phenotype hereafter referred to as Sd. However, the mechanisms underlying Sd, and the eco-evolutionary implications for host-symbiont interactions, remain poorly understood.

To address this, we first mapped the pleiotropic effects of an Sd-inducing Wolbachia infection in haplodiploid *Tetranychus urticae* mites. Using near-isogenic infected and cured lines, we quantified the impact of infection on fecundity, hatch rate, developmental time, and egg size. Maternal transmission efficiency of the Sd-inducing Wolbachia variant was also quantified. As Wolbachia-mediated effects might be contingent on host age, we tested the influence of maternal age on these measurements. Parametrized individual-based simulations were performed to evaluate these effects on the spread of Wolbachia within host

populations. We subsequently performed comparative genomics and transcriptomics to further uncover the underpinning mechanisms of Sd.

Our empirical and theoretical work show that Sd is associated with complex coordinated shifts in host life-history traits that skew sex ratios toward infected females, strongly promoting Wolbachia transmission. Of note, our simulations reveal that even modest shifts in developmental timing and hatch rate dramatically facilitate Wolbachia spread within haplodiploid populations. Additionally, genomic analyses of our Wolbachia variant are concurrent with theoretical work that predicts that Sd facilitates infection persistence upon loss of cytoplasmic incompatibility. Host transcriptomics identified several candidate genes potentially involved in Sd, providing a first insight into the molecular basis of this reproductive phenotype. Together, these experiments uncover novel mechanistic insights into how Wolbachia spread and are maintained in haplodiploid populations.



Symbiosis-mediated gene transfer in early-diverging Metazoan

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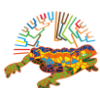
In animals, new phenotypic traits typically emerge through gene duplication, splice variation, recombination, or gene loss. Lateral Gene Transfer (LGT), the movement of genetic material across species boundaries, offers an alternative path to acquire new functions. While common in microbes, LGT is rare in animals due to germline segregation. However, animals with asexual lifestyles and diverse microbial communities, such as sponges, have increased potentials for host-microbe gene exchange.

Many sponges inhabit low-oxygen environments and display remarkable tolerance to hypoxia. Here, we identify a possible mechanism for hypoxia tolerance in freshwater sponges through anaerobic respiration. Microbial eukaryotes and some bacteria synthesize the anaerobic cofactor rhodoquinone (RQ) using the enzyme RquA. RQ facilitates electron transfer between Complex I and Complex II of the mitochondrial respiratory chain whereby fumarate, and not oxygen, is the terminal electron acceptor. We identified an ortholog of RquA in multiple freshwater sponges, likely acquired via LGT from a microbial eukaryote. The absence of RquA in marine

sponges suggests that this gene transfer event occurred in Spongillidae following their divergence from marine lineages.

Quinone profiling using liquid chromatography-mass spectrometry revealed that while marine sponges primarily contain UQs with traces of RQs, freshwater sponges exhibit significantly higher RQ levels, likely synthesized from UQ via the *rquA* gene. Exposure of adult freshwater sponges to 2% O₂ resulted in subtle *rquA* upregulation after 72 hours, suggesting a potential role in long-term adaptation to hypoxia. Phenotypic assessment with confocal microscopy showed no major structural changes under hypoxia, including the integrity of the osculum and choanocyte chambers, thus indicating strong tolerance to hypoxia.

Our results suggest that freshwater sponges have acquired a functional *rquA* gene via LGT, enabling endogenous RQ synthesis as an adaptation to low-oxygen environments. This finding expands our understanding of LGT in animals and highlights the potential role of microbial symbioses in shaping animal metabolism and ecological resilience.



The dynamics of coral symbiosis across latitude; opportunities and challenges of coral range expansion in a warming world.

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MEETING ROOM 116, August 21, 2025, 10:30 AM - 12:30 PM

The tight nutritional recycling between corals and their photosynthetic intracellular algae is among the most successful and prolific associations to have evolved as evidenced by the extensive coral reef structures within relatively nutrient poor tropical waters. Yet the warm, eutrophic, stable conditions which favoured this association is shifting. In the tropics, temperature anomalies that exceed a corals' thermal maxima have become the norm, while at poleward range edges thermally suitable habitats for corals appear to be extending, with cases of coral expansions into higher latitudes. Here we consider how the reliance of corals on their algal symbionts restricts and/or enhances their latitudinal ranges. We generated

thermal performance curves of physiological data from three coral genera and their associated symbionts collected from four locations in the Northwest Pacific. These data indicate that acclimation/adaptation to thermal extremes experienced at high latitudes may be limited by symbiont physiology, but that symbiotic associations show flexibility across the range. Furthermore, we present stable isotope data from a subset of locations to examine the role of trophic plasticity, the ability of corals to shift away from their reliance on symbiont derived nutrients, in understanding the limits on current and ongoing coral range expansion.



The role of *Wolbachia* in reproductive isolation and speciation in *Drosophila paulistorum*

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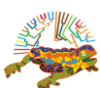
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Wolbachia is a genus of maternally inherited endosymbiotic bacteria infecting hosts of the Arthropoda and Nematoda phyla. The broad distribution and wide host range are mainly attributed to the bacterium's remarkable diversity in strategies to manipulate host biological processes, enabling it to spread into and persist in populations. Among the most well-studied phenotypes associated with *Wolbachia* infection is the ability to influence host reproduction by inducing cytoplasmic incompatibility (CI). In *Drosophila*, CI is induced by a modification of the sperm during spermatogenesis, resulting in embryonic lethality upon mating with an uninfected female (unidirectional CI) or a female infected with an incompatible *Wolbachia* strain (bidirectional CI). Viable offspring are produced only in matings with partners possessing a compatible factor to restore the modification. In populations harboring multiple, bidirectionally incompatible *Wolbachia* strains, reproductive isolation and reduced gene flow between subpopulations infected with incompatible *Wolbachia* suggest a potential role for the endosymbiont in facilitating sympatric host speciation.

The *Drosophila paulistorum* superspecies, comprising six reproductively isolated but geographically overlapping incipient species, infected with highly similar *Wolbachia* strains, offers a unique opportunity to study the speciation process and assess the role of *Wolbachia* in driving this process. *D. paulistorum* differs from its close relatives in the willistoni group by harboring an obligate, rather than facultative, *Wolbachia* infection. Moreover, *Wolbachia* is associated with both pre- and post-mating isolation in *D. paulistorum*. Reduction in *Wolbachia* titer alters male pheromone profiles critical for

semispecies-specific mate recognition and crosses over the semispecies boundaries results in hybrid lethality and male sterility. Beyond reproductive effects, *Wolbachia* impacts a broad range of biological processes, including immunity, metabolism, and muscular functions in *D. paulistorum*, indicating the impact of the infection is by no means restricted to processes related to reproduction.

Little is known about the genomic differences between *Wolbachia* infections in different species of the willistoni and saltans groups, and what designates the obligate association in *D. paulistorum*. An intriguing hypothesis is that the CI-inducing genes, *cifA* and *cifB*, present in *D. paulistorum* but absent in other members of the willistoni subgroup, contribute to reproductive isolation and consequently to the incipient speciation process. We sequenced *Wolbachia* genomes from multiple semispecies of *D. paulistorum* and related species from the willistoni and saltans groups along with their corresponding *Drosophila* host genomes. Through phylogenomics and comparative genomics, we uncover both sequence-level and structural differences in *Wolbachia* across and within species and semispecies. We analyze candidate genes involved in host interaction, investigate patterns of genome reduction associated with the obligate symbiosis in *D. paulistorum*, and assess phylogenetic congruence among *Wolbachia* and host genomes. Our aim is to provide insights into the impact of *Wolbachia* on host evolution, advance understanding of the incipient speciation process in the *D. paulistorum* superspecies, and uncover genomic features underlying the transition from facultative to obligate endosymbiosis.



Unraveling the mechanisms driving transitions to parthenogenesis in a *Wolbachia*-infected ladybug

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MEETING ROOM 116, August 20, 2025, 10:30 AM - 12:30 PM

Parthenogenesis in animals is a reproductive mechanism enabling females to produce offspring without a mate. While the evolutionary advantages behind the transition from sexual reproduction to parthenogenesis are well understood, the specific mechanisms by which transitions occur in each case remain unclear. Hybridization, infection by a parthenogenesis-inducing bacterial endosymbiont, and odd-numbered polyploidization are traits that are often associated with transitions to parthenogenesis in arthropods. With these observations in mind, we investigated the origin of the first case of parthenogenesis in a ladybug by generating a long-read genome assembly of *Nephus voeltzkowi* individuals from one of several recently discovered parthenogenetic populations to uncover the presence of any of these traits. We found a rare combination of all three traits as parthenogenetic individuals appear to be of

hybrid origin, infected with *Wolbachia* bacteria, and possess triploid genomes, whereas the sexual populations are diploid and lack these traits. We performed a *Wolbachia* elimination experiment to determine the role that the infection plays in the reproduction of the parthenogenetic *N. voeltzkowi*. The experiment demonstrated that the elimination of *Wolbachia* resulted in a reduction in egg hatching rate followed by a drastic reduction in egg production among treated females. This suggests that infection by these bacteria is necessary for successful reproduction in the parthenogenetic females and *Wolbachia* may even play a two-part role by facilitating egg laying and early embryo development. Understanding the traits underlying transitions to parthenogenesis and their relation to each other will provide new insights into the diversity, mechanisms, and evolutionary consequences of alternative reproductive modes in animals.

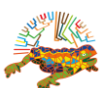




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A new method for identifying local adaptation

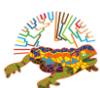
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*POSTER SESSION 1 - **P01.287**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM*

Climate change imposes novel selective pressures either by rising selection's magnitude or direction, increasing the need for robust methods to identify populations and traits keeping up with these changes. Studying current and past local adaptation will allow us to draw hypothesis on what types of traits are able to adapt. Accurate differentiation between adaptive and neutral evolutionary processes is therefore essential for creating the background necessary predictive evolutionary genomics. On that aim, we introduce LAVA, a novel statistical approach that uses estimates relatedness through allele-sharing in genome-wide markers, carefully integrated into a genetic population model to overcome existing limitations in quantifying adaptive divergence. While traditional QST-FST

comparisons fail to account for complex demographic histories and deviations from the island model, thereby generating inflated Type I error rates, LAVA incorporates between- and within-population relatedness matrices to include demographic structure. Through comparative analysis with previous methods and using simulated data under both neutral conditions and various selective regimes, we demonstrate that LAVA maintains appropriate statistical calibration across diverse population structures while keeping high statistical power to detect adaptive divergence. We further validate LAVA's relevance through a meta-analysis of empirical datasets spanning multiple taxa, revealing patterns of trait responses to selection.



A Tale of Two Seas: Local Adaptation and Osmoregulation in the copepod *Acartia tonsa*

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POSTER SESSION 1 - **P01.269**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

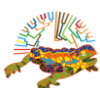
Understanding the genomic and physiological basis of local adaptation is essential for predicting species' responses to environmental change. Salinity—a key environmental factor shaping marine biodiversity—is rapidly shifting due to climate change, especially in marginal seas like the Baltic. Yet, the mechanisms enabling species to tolerate and adapt to novel salinity regimes remain poorly resolved. Here, we investigated how populations of the calanoid copepod *Acartia tonsa*, a marine zooplankter with broad salinity tolerance, persist and locally adapt across the steep salinity gradient of the North and Baltic Seas.

We used a common-garden design combined with whole genome sequencing (WGS) to test for local adaptation of copepods collected from 14 locations spanning a salinity gradient from marine in the North Sea to near freshwater conditions in the northern Baltic Sea. We quantified survival and metabolic rates under low-salinity stress and analyzed gene expression profiles to identify transcriptomic mechanisms driving salinity tolerance.

There was clear genetic differentiation between North and Baltic Sea populations,

with evidence of additional structure within the Baltic. Physiological and transcriptomic data support this pattern: Baltic copepods showed a higher capacity to survive and tolerate low-salinity stress compared to North Sea animals with a more stringent gene expression response and an osmoregulatory strategy driven by active transport processes. Together, these findings suggest that *A. tonsa* has not only colonized the Baltic Sea successfully, but has also undergone population-specific adaptation to its low salinity conditions.

By integrating genomic, transcriptomic, and physiological data, our study highlights the importance of multi-faceted approaches to uncover the mechanisms of adaptation to environmental stressors. These insights are critical for predicting species' resilience to ongoing climate-driven changes in salinity, particularly in coastal and estuarine systems where shifts are expected to be most pronounced. Here, *A. tonsa* serves as a valuable model organism for understanding evolutionary potential and vulnerability in widespread marine species under pressure from rapid environmental change.



Acute heat stress results in sex-specific mortality in birds

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POSTER SESSION 1 - **P01.281**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

As temperatures reach unprecedented highs due to global warming, understanding of mechanistic basis for adaptability to heat stress is essential to predict the impact of extreme temperature events on the persistence of animal populations. The overarching goal of this study was to understand the sex-specific effects of acute heat stress on physiological blood measures and cellular/molecular damage. Zebra Finches (*Taeniopygia guttata castanotis*), desert-adapted Australian birds, provide an ecologically relevant study system. Stress resistance has been suggested to be sex-biased with possible links to homogamety/heterogamety of the sex chromosomes and differential hormone expression. As the heterogametic sex, we hypothesized that females (ZW) would be more susceptible to damage from heat stress than males (ZZ).

We conducted an acute heat stress experiment using zebra finches bred at Auburn University, that were all adults under one-year-old. Male and female birds were assigned to two groups: Control, maintained at 22.5°C, and Acute Heat, exposed to 43°C for 5 hours. Baseline blood samples were taken two weeks prior to treatment and blood/tissues were collected immediately after treatment. Fresh whole blood was used for hematocrit, glucose, and ketone measures. DNA from blood cells was used to quantify telomeres and mtDNA copy number by quantitative real-time PCR. Telomeres are known biomarkers of acute and chronic stressors, having demonstrated to be predictors of longevity in the species. Mitochondrial DNA copy number is predictive of metabolic capacity and autophagy in response to mitochondrial damage. Specifically, we predicted that telomere lengths would be shorter and mtDNA copy number lower in females prior to heat exposure if they are more vulnerable to the stressor.

Despite the treatment of 43°C for 5 hours reported to be non-lethal in the literature, in this experiment we observed 12.5% mortality (11 birds) from this acute heat stress treatment. Only female birds died. Upon closer inspection of the data, we observed that all but one of the birds that died were from a “younger” cohort of adults (4-6 months old), relative to a slightly older cohort of adults (9-11 months old). We generated model-predicted probabilities for mortality and found both sex and age-category to be significant predictors (P-value < 0.05), with young females having 22.2% predictive probability of mortality and older females only 4.3%, while males of both age categories have near zero predictive probability of mortality. To test if molecular biomarkers taken at baseline were predictive of mortality due to acute heat, we compared young heat stressed females that did and did not die. Preliminary analysis suggests none of the baseline molecular biomarkers were predictive of mortality.

Our unexpected mortality results indicate that young adult females are the most susceptible group of young adults to heat stress, which could have important consequences on future recruitment into the population under climate change scenarios. This finding emphasizes the importance of studying the effects of stress across sexes and age classes to understand the impact of extreme heat events on population viability. However, this was a lab experiment on captive reared birds. Further research is needed to determine if these results are replicable in lab and field populations. Additional research is underway in our group to examine how males and females differed in cellular damage and repair processes that may contribute to the sex-biased mortality. This research brings new insight to sex-biased response to heat stress in birds.



Adaptive and non-adaptive responses to metal contaminants and pathogens in wild brown trout *Salmo trutta*

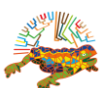
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POSTER SESSION 1 - **P01.257**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Multiple stressors are leading to major changes in rivers. Among them, the interaction between chronic pollution and emerging pathogens remains poorly known. Moreover, we do not know whether wild fish populations could show adaptive divergence in response to this combination of stressors. In a collaborative project, we investigated the evolutionary responses of wild brown trout *Salmo trutta* to combined metal contamination and/or exposure to the emerging pathogen *Tetracapsuloides bryosalmonae* responsible for the highly lethal PKD disease (Proliferative kidney disease) in Europe. We sampled 40 fish populations along gradients of metal pollution from historic mining and parasitism risk (variations of parasite prevalence and load). Using 192 SNPs, we compared the genetic diversity and genomic structure and measured fish phenotypic traits such as morphology,

coloration, body condition, and immunity indicators to measure their genetic and phenotypic divergence and health status. Results show a phenotypic divergence on several traits linked to variations in metal contamination (especially by Pb, Cd and Cu). due to mining, even centuries after the closing of the mines. To investigate the evolutionary mechanisms leading to such divergence, we then used a Pst-Fst approach, suggesting that multiple stressors could be a selective driver of phenotypic divergence in mountainous rivers. We also used a GEA analysis (Genome-Environment association) to characterize main SNPs involved in response to pollutants (preliminary results). With this study, we hope to encourage future studies in the growing field of evolutionary ecotoxicology, to better forecast the consequences of global change and multiple stressors on the evolutionary trajectories of wildlife.



An evolutionary and mechanistic study of stressor interaction patterns between pesticides, fish predation and heatwaves in natural populations of *Daphnia magna*

Li J¹

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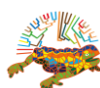
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POSTER SESSION 1 - P01.258, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Because of global change, species are increasingly facing a multiple-stressor world. This is especially concerning as global change stressors may magnify each other's negative effects. Notably, such stressor interactions cannot easily be predicted by the effect of single stressors, highlighting the crucial need for explicit multiple-stressor research to avoid so-called ecological surprises. Several knowledge gaps remain to advance multiple-stressor research and to transform it into a more predictive discipline, which is crucial to integrate it into risk assessment frameworks. Therefore, I will fill these knowledge gaps by investigating how natural populations of *Daphnia magna* respond to pesticide pollution, fish predation, and heat waves. Firstly, I will research the evolution of natural population tolerance to pesticides, fish predation and their interacting response patterns through common-garden exposure experiments and

explore how natural populations of *Daphnia magna* are adapted to alternating between combined and single stressors through ecotoxicological experiments. Secondly, I look for 'super clones': I expose clones with strong 'pesticide and fish' resistance to heatwaves, to obtain natural populations that are resistant to a wide range of stressors. Meanwhile, I focus on the transcriptome's role in shaping tolerance in *Daphnia magna*. I will be sequencing RNA to explore the types of interactions at the gene level and the regulation of biological pathways for natural population fitness in *Daphnia magna*. This research will contribute to our understanding of patterns of stressor interactions and the evolution of adaptations in natural populations.

KEYWORDS: Evolution; Adaptability; Multiple stressor; Transcriptome; *Daphnia magna*



Biogeographic and adaptative history of the butterfly *Heliconius erato* in Neotropical forests: understanding the past to preserve the future

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POSTER SESSION 1 - P01.268, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The evolutionary history of the Neotropical region over the last 250 million years is related to a succession of major geological events that altered changed the continents and ocean basins, as well as the climate and evolution of species. The Brazilian rainforests experienced undergone these events, including the Miocene uplift of mountains, changes in the course of rivers in the Amazon, and the climatic oscillations of the Pleistocene.

These processes, such as the uplift of the mountains, rivers and other geographic barriers, and climate fluctuations, contributed to the great diversity found in the Amazon and Atlantic Forests. On the other hand, the climate changes taking place in the Anthropocene may not be a “generator of diversity” but rather of extinction. The process of evolution is not occurring at the same rate, resulting in a loss of genetic diversity, generating a decrease in the adaptive response of species.

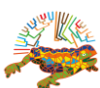
The use of molecular tools has allowed us to better understand how climatic and geological events and changes in the landscape influence the diversification and demographic, evolutionary, and adaptive processes of natural populations. In this work we are using SNPs (single nucleotide polymorphisms) to identify possible neutral and adaptive polymorphisms in populations of the butterfly *Heliconius erato* (Nymphalidae: Heliconiini), a widely distributed Neotropical butterfly, occurring in several

biomes including the Amazon, Atlantic Forest, Cerrado, and Pampa.

We aim to: (i) analyze the structure of the genetic diversity in *H. erato* across its geographic range; (ii) investigate genomic signatures that indicate historical connections between the southern Atlantic Forest and western Amazon, as well as between the northern Atlantic Forest and eastern Amazon; (iii) identify genes associated with local adaptation in response to climatic variation; and (iv) assess how both neutral and adaptive genetic variation may respond to future climate change scenarios. A total of 276 individuals from 47 localities were sampled, representing the most comprehensive sampling to date for investigating the proposed objectives and hypotheses.

Butterflies are organisms considered to be environmental bioindicators, and the presence or absence of certain species in certain areas can be indicative of the state of conservation of an environment. *Heliconius erato* is a widely distributed species, found in different environments, from conserved areas to urban areas. If our results show that this very common species will lose genetic variability, what can be expected of restricted species?

The expected results will contribute to a deeper understanding of the processes that have shaped Neotropical biodiversity and how species may respond to climate change over time.



Bridging developmental plasticity and climate adaptation through evolutionary genomics, population genetics and transcriptomics in African *Bicyclus* butterflies

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POSTER SESSION 1 - P01.291, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The climate crisis endangers much of our world's biota, urging the need for an in-depth understanding of species' adaptive potential. Phenotypic plasticity, the expression of multiple phenotypes from a single genome, is a well-studied adaptation to short-term environmental fluctuations, but its contribution to long-term adaptation remains contentious. In seasonal environments, phenotypic plasticity is often triggered during development, when individuals integrate environmental cues into molecular pathways that will later shape their adult traits. With increased climatic stochasticity, these finely tuned cascades might lead to mismatches between the environment and the expressed phenotype. The risk for wild populations is that new responses cannot evolve rapidly enough, due to insufficient genetic variation in core regulators or due to developmental constraints.

Our research bridges evolutionary developmental biology, population genetics, and conservation genomics to investigate the molecular basis and adaptive potential of seasonal plasticity in *Bicyclus* butterflies, a diverse African genus that exhibits differences in seasonally-driven developmental responses. In some species, such as the lab-adapted *Bicyclus anynana*, seasonal morphs are highly distinct: temperature cues during development determine whether individuals develop into short-lived, reproductive wet-season forms or long-lived, inactive dry-season forms. In other species across the phylogeny, phenotypic plasticity is either reduced or absent. Here, we combine wet-lab developmental experiments with whole-

genome and population genomic approaches in wild *Bicyclus* species to understand how developmental plasticity contributes to climate adaptation. More precisely, we (i) characterise the timing of the developmental window during which environmental cues influence phenotype in *Bicyclus anynana*, demonstrating that phenotypic plasticity is restricted to a narrow period from the late larval stage to early pupation; (ii) apply transcriptomics (RNA-seq) and chromatin accessibility profiling (ATAC-seq) to map the gene regulatory networks that establish alternative seasonal forms in this species; (iii) leverage the diversity of plastic strategies across the *Bicyclus* phylogeny to (a) test how plasticity levels shape intraspecific variation in core developmental regulators, and (b) identify genomic features associated with plasticity, generating 14 high-quality de novo genome assemblies and integrating these with population resequencing data; and (iv) assess how selective pressures on these regulatory elements are driven by ecological gradients using 6 populations in 3 habitats from 3 focal species in Cameroon, to help predict their adaptive potential under climate change.

Together, this research provides an integrated developmental and evolutionary framework for understanding how phenotypic plasticity evolves and supports adaptation in the wild. It generates valuable genomic resources for understudied African butterflies, informs conservation strategies under Anthropocene-driven pressures, and advances broader efforts to uncover the molecular basis of adaptation in biodiversity hotspots.



Cold-response is altered by salinity exposure in an intertidal snail

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POSTER SESSION 1 - P01.267, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Melampus is a genus of air breathing estuarine snails that are capable of high dispersal via planktonic larvae. Three cryptic species are found in Spartina dominated marshes on the Atlantic coast of North America, where they have distinct but patchily overlapping ranges. It is not clear what determines the areas of species co-existence, but ecological niche modelling suggests that ocean salinity and temperature differ subtly between their respective geographic ranges. To explore the genetic basis of response to these interacting stressors, we acclimated *Melampus jaumei* to different salinities (5, 20, and 35ppt) followed by an acute cold shock.

We used transcriptomic and metabolomic comparisons in the same individuals to show that salinity exposure markedly changed cold response. This has also helped identify putative genes associated with freeze tolerance through the initiation of ice formation (ice nucleation). Since these snails live in tidal estuaries with strong salinity gradients and highly fluctuating conditions, this suggests that subtle differences in their response to interacting stressors could explain large scale geographic variation among species. It also highlights the importance of preserving entire estuarine habitats: the highest intertidal zones occupied by these species are frequently eliminated by sea wall construction, which will effectively reduce both species diversity and the physiological variation that accompanies this.



Conservation genomics of the threatened boreal wood-decay fungus *Amylocystis lapponica*

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POSTER SESSION 1 - P01.293, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Old-growth forests are declining due to intensive forestry activities. This has significantly reduced the availability of dead wood substrates that are essential for the survival of many saproxylic organisms. Wood decay fungi are the main decomposers of dead wood and play a pivotal role in the carbon cycle and nutrient cycling in boreal forests. They rely on a continuous supply of suitable dead wood that they can colonize, decompose, and eventually leave once the resources are depleted. *Amylocystis lapponica* is a red-listed, wood-decaying polypore fungus with a strong affinity for undisturbed, late-successional forests. It likely expanded its range across northern and eastern Europe following the postglacial colonization of its main host, *Picea abies*. In this study, we investigate the genetic structure and levels of genetic variation in *Amylocystis lapponica* across populations in northern Europe, focusing on how forestry practices may have influenced these patterns. To address this, we assembled a broad collection of *A. lapponica* isolates and conducted population genomic analyses based on SNP data.

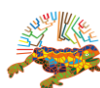
Materials and methods: A total of 48 fruit bodies of *Amylocystis lapponica* were collected from old-growth forests in six Northern European countries: Slovakia, Russia, Poland, Sweden, Finland, and Norway. DNA was extracted and SNP data were generated to study genetic variation. We analyzed population structure using both admixture modeling and principal component analysis, and tested for isolation by distance by comparing genetic and geographic distances between populations. Genetic diversity within

populations was measured using standard statistics, and signs of historical population expansion or contraction were assessed using Tajima's D. Lastly, we examined potential signals of local adaptation to environmental and habitat variables using redundancy analysis (RDA) and association testing with *Samβada*, and related genetic patterns to forest characteristics such as habitat size, isolation, and forest management history.

Results: Our results suggest that *Amylocystis lapponica* likely represents a single panmictic lineage across its Northern European range, in line with expectations of a postglacial expansion from a single refugium. The lack of population structure also indicates that habitat fragmentation reduced population size resulting from human activities has not been strong enough to result in genetic structuring. Further demographic analyses are ongoing to assess whether there has been an overall reduction in population size.

RDA identified several loci potentially associated with climate variation across the study area. This suggests that climate may exert selective pressure on *A. lapponica*, leading to evolutionary differences in allele frequencies along environmental gradients. Functional annotation and further investigation of the adaptive significance of these loci are currently underway.

Conclusion: *Amylocystis lapponica* likely forms a single panmictic lineage across Northern Europe, consistent with postglacial expansion. Climate-driven selective pressures may influence genetic variation, with ongoing research exploring the adaptive significance of the identified loci.



Convergent molecular evolution of thermal tolerance in mammals

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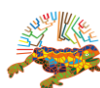
¹School of Life Sciences, Faculty of Medicine and Health Sciences, University Of Nottingham, ²Discovery and Translational Sciences Department, Leeds Institute of Cardiovascular and Metabolic Medicine, Faculty of Medicine and Health, University of Leeds, ³School of Medicine, University of Leeds, ⁴LeedsOmics, University of Leeds

POSTER SESSION 1 - P01.261, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Mammals vary in their ability to tolerate heat stress, a trait of great relevance to fitness in the current context of global climate change. For model mammal species such as mice, pigs and cattle, we have gained some specific insights into molecular responses to heat stress, but broader patterns across the wide diversity of the mammal phylogeny are not known. Right across the mammal phylogeny we observe multiple independent emergences of heat tolerant lineages. We wish to determine whether this convergent trait is underpinned by molecular adaptation.

We have assembled a dataset encompassing 230 mammal species for which we have access to annotated

genomes. These species include pairings of close relatives that differ strongly in typical maximum environmental temperatures encountered. For example, we categorise the lion *Panthera leo* as heat adapted as it occupies a geographical range with a typical maximum temperature of 35°C, whereas its paired species, the snow leopard *Panthera onca* (diverging ~9 Million Years Ago) encounters maximum temperatures ~16.5°C. Using branch-site models, we will identify genes with significant variation in selective pressure across these pairings. Comparing results across all convergent instances allows us to identify key genes, pathways and processes common to heat tolerant species.



Disentangling the genomic basis of terrestrialization across animals through a transcriptomics spyglass

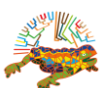
Eleftheriadi K¹, Salces-Ortiz J¹, Escudero N¹, Vargas Chavez C¹, Fernández-Álvarez F², Fernandez R¹

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POSTER SESSION 1 - **P01.250**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Terrestrialization, the colonization of land by aquatic animals, plants, and fungi, is one of the most remarkable events in the history of life on Earth. From a stable marine environment, animals altered respiration, excretion, reproduction, etc. to adapt to life on land. Of the 36 animal phyla, 9 undoubtedly include terrestrial forms. The genomic basis of this transition remains largely unexplored. To uncover the genes that facilitated this adaptation, we subjected terrestrial animals and their aquatic relatives in each phylum to 12 experiments mimicking abiotic stressors associated with land colonization (e.g., visible light and UV exposure, chemosensory cues, and changes in oxygen concentration and osmoregulation). These experiments, involving 23 species from 9 phyla, yielded

~1,000 transcriptomes sequenced using Illumina and PacBio Iso-Seq. We employed comparative transcriptomics, gene co-expression networks and phylostratigraphy to investigate the genomic toolkit underlying terrestrialization, as well as the evolutionary origin of its genetic components. Our results revealed that, while genes involved in responses to abiotic stress in both marine and terrestrial species emerged at similar evolutionary time points, they often belong to different orthogroups, indicating lineage-specific functional diversification. Key lineage- and phylum-specific genes and modules involved in these adaptations were identified. This study provides novel insights into how animals convergently reshaped their genomes to overcome the physiological and morphological challenges of life on land.



Environmental adaptations in *Arabidopsis lyrata*

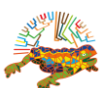
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¹Max Planck Institute For Plant Breeding Research

POSTER SESSION 1 - P01.254, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Environmental adaptation in plants involves complex genetic mechanisms that enable survival and reproduction across diverse habitats. Understanding these mechanisms is crucial for uncovering how plants adapt to their ecological niche. *Arabidopsis lyrata* has successfully colonized high latitudes, making it an ideal model for studying patterns of environmental adaptation. Its natural range provides a unique opportunity to investigate the genetic basis of adaptation to diverse climatic conditions such as cold temperatures and high UV radiation. Using

genome-wide association studies (GWAS), we analyzed diploid populations of *A. lyrata* to identify loci associated with latitudinal variation. Our analysis highlights genes linked to critical traits, including flowering time regulation, photosynthesis and soil adaptation, which may be adaptive at high latitudes. These findings reveal key genetic loci that contribute to environmental adaptation in *A. lyrata*. Moreover, our results suggest *A. lyrata* as a promising model for further GWAS studies.



Environmental insights into sex chromosome evolution in a sex-reversing reptile (*Carinacincus ocellatus*)

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¹University of Tasmania, ²University of Montpellier

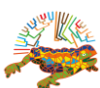
POSTER SESSION 1 - **P01.282**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Climate change is threatening many species with extinction, and clear examples are provided by those whose sex is determined by temperature, which may experience future sex ratio biases that threaten population persistence. Species in which sex is determined genetically—by sex chromosomes—were thought immune to these problems. However, examples are accumulating of temperatures overriding genetic sex determination, generating so-called ‘sex-reversed’ individuals.

The viviparous Tasmanian spotted snow skink, *Carinascincus ocellatus*, provides one such example. In this species, climate-induced selection may also be evidenced by geographic variation in the frequency of sex reversal. Concomitantly, the magnitude of sex chromosome differentiation appears higher where sex reversal is rare, as would be expected with increasing genotypic-phenotypic sex mismatch reducing individual fitness. However, the possible selective links between climate, sex reversal, and sex chromosome differentiation has only been established based on observations

from two *C. ocellatus* populations. Here we tested more broadly whether sex chromosome differentiation is greater in colder and less variable climates (i.e., higher elevations), where observations and theory for *C. ocellatus* suggest sex reversal is less likely. We obtained genetic samples from 251 individuals across nine populations spanning the species' altitudinal range and using DArT-seq, a genome complexity reduction-based sequencing technology, we characterized the number and distribution of sex-linked loci across populations.

Our results are not consistent with an increase in sex chromosome differentiation with elevation, and hence climate. This could reflect the possibility that selection against sex reversal has not yet manifest via sex chromosome differentiation at the additional cold climate sites we analysed herein, reflecting localised emergence at one high elevation site and/or genetic isolation among such sites. Future research should quantify rates of sex reversal in additional cold (and warm) climate populations to confirm that our assumptions of sex.



Evolution of facultative symbiosis in the stony coral *Oculina*

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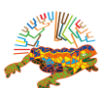
POSTER SESSION 1 - P01.286, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Most stony corals are obligate symbionts, dependent on nutrients provided by the photosynthetic activity of dinoflagellates residing within specialized cells. Disruption of this symbiotic consortium leads to coral bleaching and, ultimately, mortality. However, a few coral species exhibit facultative symbiosis, allowing them to survive extended periods of bleaching. Despite this resilience, the underlying biological mechanisms remain poorly understood. Here, we investigate the genomic and cellular basis of facultative symbiosis in *Oculina patagonica*, a thermotolerant Mediterranean coral.

Methods: We have sequenced and annotated a chromosome-scale genome of *Oculina patagonica* and built single-cell transcriptomic atlases for this species—including symbiotic

and aposymbiotic specimens—and two obligate symbiotic corals.

Results and conclusions: Comparative genomic analysis revealed karyotypic and syntenic conservation across all scleractinians, with species-specific gene expansions primarily driven by tandem duplications. Single-cell transcriptomic profiling of symbiotic and naturally aposymbiotic wild specimens identified an increase in phagocytic immune cells and a metabolic shift in gastrodermal gene expression from growth-related functions to quiescent, epithelial-like states. Cross-species comparison of host cells uncovered *Oculina*-specific metabolic and signalling adaptations indicative of an opportunistic, dual-feeding strategy that decouples survival from symbiotic state.



Evolutionary Tuning: Understanding the Influence of Stage-Specific Diet in Shaping Developmental time in *Drosophila melanogaster*

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POSTER SESSION 1 - **P01.255**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Organismal development is influenced by both genetic and environmental factors, with nutrition playing a pivotal role in shaping growth, physiology, and reproduction. Adequate nutrition during development is essential for optimal growth, physiological development, and cognitive function. Conversely, poor nutrition during development can slow or arrest growth, impair physiological function, and ultimately reduce fitness. Studies indicate that adaptation to poor nutrition during development can lead to accelerated development, impacting body size and fertility. However, an organism's life cycle is not defined solely by developmental stage; it also involves critical reproductive stages. Nutritional requirements during these two stages can differ based on their physiological demands. During the developmental stage, nutrients support the growth and development of organs, while in the reproductive stage they facilitate reproduction and survival. This stage-specific allocation of nutrients plays a crucial role in energy investment, shaping an organism's fitness and its evolutionary success. While adaptation to developmental nutrition is well established in affecting organismal development, relatively less is known about how nutritional adaptation during both developmental and reproductive stages can cross-talk over evolutionary timescales to drive pre-adult development and their fitness costs. To address this gap, I used a large outbred population of *Drosophila melanogaster* due to its distinct developmental (larval) and reproductive (adult) stages, conserved nutrition-

responsive pathways, and short generation time, facilitating evolutionary studies. Recent research on nutrition suggests that not just calories but macronutrient ratios modulate health in several model organisms, including *Drosophila*. Hence, I am using isocaloric macronutrient diets for both larval and adult stages. The treatments consisted of two isocaloric diets with varying protein-to-carbohydrate ratios: a carbohydrate-rich (C) and a protein-rich (P) diet for both life stages in a full-factorial design, generating four regimes (PP, PC, CC, CP); the first letter denotes the larval diet, and the second letter denotes the adult diet. To assess the long-term effects of stage-specific dietary effects, I employed experimental evolution for the above regimes, with four independent replicate populations for each. These populations were maintained for 59 generations under selection, followed by two generations of standardization in the ancestral environment (N) to remove the non-genetic effects. Our results show that flies evolved on a carbohydrate-rich larval diet had accelerated development in the ancestral environment compared to those evolved on a protein-rich larval diet. Interestingly, adult diet also influenced pre-adult developmental time, with flies selected on a carbohydrate-rich diet in the adult stage developing slower in the ancestral condition compared to flies on protein-rich adult food. These findings highlight novel insights that selection under both developmental and adult diet influences the pre-adult development in *D. melanogaster*.



Fecal metagenomics as a conservation tool: Assessing pathogen loads in the critically endangered Iberian desman

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POSTER SESSION 1 - P01.256, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Infectious diseases are a major threat to the survival of wild populations, especially in endangered species, where their effects can be amplified by environmental stressors and population fragmentation. Moreover, wildlife is recognized as an important reservoir of zoonotic pathogens that can pose health risks not only to animal populations but also to humans. Recent advances in metagenomic analysis of fecal samples offer a powerful, non-invasive tool to assess the diversity and dynamics of pathogens and parasites that may threaten population viability. However, accurate identification of these infectious agents remains challenging due to the complexity of microbial communities and limited reference genomes. To address these methodological issues, we developed a novel computational framework for detecting potentially pathogenic bacteria from metagenomic sequences. Several key parameters affecting the analysis, including mapping algorithm, database configuration, and alignment-

derived metrics, were optimized to improve identification sensitivity and specificity. Applying this approach to fresh fecal samples from a population of the Iberian desman (*Galemys pyrenaicus*), a critically endangered semi-aquatic mammal, we identified multiple potentially pathogenic bacterial species with varying prevalences within the population. Furthermore, our analysis revealed some desmans with elevated or altered pathogen loads, suggesting variations in environmental exposure, health status, or underlying genomic susceptibility, which opens new research avenues for understanding host-pathogen-environment interactions. From an applied perspective, our study demonstrates a novel approach for species-level pathogen detection using non-invasive fecal metagenomic analysis, providing essential support for population management and the development of more effective conservation strategies.



Gene expansions in herbivorous rodents drive rapid dietary adaptation to climate change

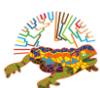
Dearing M¹, Klure D¹, Greenhalgh R³, Shapiro M¹, Orr T²

¹University Of Utah, ²New Mexico State University, ³St. Jude's Children's Research Hospital

POSTER SESSION 1 - **P01.279**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Herbivores routinely feed on plants with bioactive compounds, and in doing so ingest substantial quantities of toxins. How mammalian herbivores have evolved to feed on chemically defended plants and the particular physiological mechanism used is poorly understood. We investigated the evolution of dietary adaptation in mammalian herbivores by focusing on two woodrat species (*Neotoma lepida* and *N. bryanti*) that independently experienced a climate-related event with significant dietary consequences — the extirpation of their primary food resource, juniper (*Juniperus* spp.) and its replacement by creosote bush (*Larrea tridentata*). At the end of the Last Glacial Maximum (~19–15 kya), juniper was extirpated at low elevations of the American Southwest as the region underwent a natural warming event that led to the invasion of creosote bush, a novel species. Juniper and creosote bush produce disparate plant secondary compounds that require different suites of biotransformation enzymes. Creosote leaves are heavily defended by a complex resin of hundreds of chemicals (primarily phenolics, lignans) in substantial quantities comprising 10–25% of its dry mass. We surveyed both species of woodrats across a 900 km transect and combined whole-organism performance assays on

creosote resin feeding capacity, gene induction experiments and population genomics to characterize the genomic architecture and physiological processes that enable these woodrats to feed on creosote bush. We found that creosote-adapted woodrats have greatly elevated gene dosage within multiple biotransformation enzyme families such as P450 cytochrome monooxidases and UDP-glucuronosyltransferases. These duplication events have occurred independently across species and substantially increase expression of biotransformation genes, especially within the glucuronidation pathway. In addition, these parallel expansion events drove copy number of genes involved in one detoxification pathway — the glucuronidation pathway — to substantially high levels. Moreover, copy number variation within key detoxification gene families closely mirrors the historic expansion of creosote bush across the American southwest such that woodrats with longer exposure to creosote bush have higher gene copy number. We propose that duplication resulting in increased gene dosage is an important mechanism by which animals initially adapt to novel environmental pressures.



Genetic Diversity and Population Structure of Bolivian Camelids Revealed by SNP Genotyping

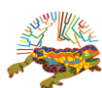
Roman Peña A¹, Strandberg E¹, Johansson A¹, Iñiguez V², Barreta J², Chiri R³, Saavedra V, Gutierrez L³, Periasamy K⁴

¹Swedish University Of Agricultural Sciences, ²University Mayor de San Andres , ³Technical University of Oruro, ⁴International Atomic Energy Agency

POSTER SESSION 1 - **P01.295**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The South American camelids (llamas, alpacas, vicuñas, and guanacos) play a crucial role in Bolivia's economic and cultural landscape and the wider region. These species support the livelihoods of many indigenous communities by providing fiber for textiles, meat, and transportation in high-altitude environments. Understanding their genetic diversity and population structure is essential for effective conservation and management strategies. Previous studies using microsatellites and mitochondrial markers have reported high genetic diversity and introgression in alpacas and population stratification in Bolivian vicuñas. In addition, levels of introgression suggest the need to analyze hybrid individuals between the domestic camelids (llamas and alpacas). In this study, we aimed to evaluate the population structure and genetic diversity within and between camelid species populations in the Bolivian highlands using SNP genotyping data. The study included llamas (N = 177), alpacas (N = 102), vicuñas (N = 103), and hybrids (N = 16). DNA genotyping was performed using the Axiom Camelids Genotyping Array (alpaca) from Affymetrix, containing 59,995 SNPs. After quality control, the final dataset comprised 46,125 SNPs across 370 individuals. Runs of homozygosity (ROH) and principal component analysis (PCA) were conducted using PLINK v1.9. Admixture analysis was performed with the LEA package in R, applying a Bayesian-based model and

testing K values (number of latent factors) from 1 to 10. The results indicated that hybrids and alpacas exhibited significantly higher heterozygosity ($p < 0.05$), as evidenced by lower FROH values (means = 0.09 and 0.10, respectively), compared to llamas (mean = 0.17) and vicuñas (mean = 0.18). These findings suggest greater genetic diversity in alpacas, consistent with the admixture analysis results. The PCA revealed that the first principal component explained 31.5% of the genetic variation among species. Evidence of introgression between domestic species was supported by six hybrids clustering with either llamas or alpacas and three llamas clustering with alpacas or hybrids. Bayesian clustering across all species indicated an optimal $K = 2$, showing admixture in alpacas and hybrids between llamas and vicuñas. Within-species PCA for alpacas and llamas showed slight stratification, with two principal components explaining less than 5% of the variation. For vicuñas, PCA and admixture analysis revealed clear stratification associated with geographic distribution, with more pronounced clustering between the two subspecies than previously expected. The admixture results highlighted a strong geographic structure in vicuñas, in contrast to the domestic species, and the elevated FROH values support limited gene flow and may reflect a historical population bottleneck.



Genomic analysis reveals local adaptation and vulnerability to climate change of the African orphan crop finger millet (*Eleusine coracana*)

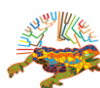
Veltman M¹, Thuillet A, Barnaud A, Vigouroux Y

¹Institut De Recherche Pour Le Développement

POSTER SESSION 1 - P01.292, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

An important crop in arid and semi-arid regions, finger millet is valued for its nutritional composition, offering high concentrations of fibers and micronutrients, and its climate resilience, exhibiting improved biotic and abiotic stress tolerance compared to major crops. These properties have earned finger millet the reputation of a climate-smart "super food" that promises to combat food insecurity in parts of the world that suffer from malnutrition and reduced yields due to the effects of climate change. Despite this great potential, evolutionary studies investigating the genetic basis of climate adaptation in finger millet have not been carried out to date. Here we aim to fill this gap by providing a reference panel of more than 200 finger millet accessions sampled across its cultivation range in Africa and India. By analysing genome-wide

patterns of diversity and environmental selection, we identify signatures of local adaptation and their likely environmental drivers. Using predictions of future climatic conditions based on different emissions scenarios and climate models, we map the possible consequences of climate change for genome-environment associations that evolved over millennia, and identify areas where maladaptation to the environment may occur as a result. Finally, we leverage the range of diversity and adaptive potential present in this reference panel to identify candidates for assisted migration into (and out of) areas that are especially vulnerable to climate change. This could not only mitigate potential yield losses and reductions in cultivation area, but also safeguard the precious genetic resources of this underutilised crop for future use.



Genomic signatures and demographic history of the widespread and critically endangered yellow-breasted bunting

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POSTER SESSION 1 - **P01.259**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Population declines may have long-term genetic consequences, including genetic erosion and inbreeding depression, which could affect species' evolutionary potential and increase their risk of extinction. Small populations are more vulnerable to genetic threats than common species, but even species with large populations can also be at risk of extinction. The yellow-breasted bunting (*Emberiza aureola*) is a common and widespread songbird in the northern Palearctic regions, but its global population size has drastically declined by around 90% throughout the past 30 years, leading to an upgrade of its conservation status to critically endangered in the IUCN Red List. In this study, we identified three populations within this species using whole-genome resequencing data, but the genetic differentiation between populations was shallow. These populations underwent similar population fluctuations but differed

in the extent of population decline, resulting in lower genetic diversity and more homozygous deleterious mutations in a population comprising individuals on islands. The ancient demographic history was mainly associated with the climate, while population declines over the past 100 generations are likely due to human activities. Our results suggest that the yellow-breasted bunting population before the recent collapse faced relatively low genetic threats and had high evolutionary potential. However, we should be vigilant about the genetic threats faced by this species, as our sampling time occurred at the onset of its recent global population collapse. This study provides valuable genetic information for the conservation of yellow-breasted bunting and also highlights the similar genetic threats faced by other large populations.



Genomics of Brazilian howler monkeys reveals recent inbreeding and species-specific positive selection

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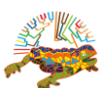
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POSTER SESSION 1 - P01.275, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Howler monkeys (genus *Alouatta*) are an understudied neotropical primate with a broad geographic distribution across Central and South America, and a wide radiation including between 9-14 species. In parts of their range, particularly in the Atlantic Forest of Brazil, rising deforestation and human encroachment into forests threaten these vulnerable species and increase human-primate interactions. Further, howler monkeys serve as primary reservoirs for multiple zoonotic malaria parasites and are highly susceptible to sylvatic yellow fever. We conducted whole-genome sequencing of 88 howler monkeys (average coverage ~11X) to reconstruct the evolutionary history of multiple howler monkey groups from across Brazil. Genomic evidence of population structure – based on PCA, ADMIXTURE, and *Fst* analyses – suggests different classifications for four major species groups, contrary to current IUCN taxonomic standards, and shows allopatric speciation increases towards the mouth of major Amazonian rivers in concordance with the

Riverine Barrier Hypothesis. We find regional differences in demography, including an increased proportion of long ROH and decreasing population size estimates, which suggest recent inbreeding in Atlantic Forest howler monkeys, potentially resulting from the increased habitat fragmentation in the Atlantic Forest compared to the Amazon rainforest. Finally, we compared signatures of positive selection using G1 statistics; in contrast to most primate studies representing a single species per genus, we tested for shared and unique adaptive histories within a genus. We found enrichment for genes relating to immune response and howler monkey-specific phenotypes like trichromatic vision and folivory, though the genomic regions were species-specific. This evidence for host adaptation to local pathogens aligns with the known burden of infectious disease, particularly in the Atlantic Forest, and is concordant with recent independent histories of the species in different environments.



Homology-Based Mapping of microRNA Evolution Across Mammals

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POSTER SESSION 1 - P01.288, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: The evolutionary history of mammals reflects a continuous process of genetic innovation that has produced remarkable biological diversity. While much effort has focused on understanding the evolution of protein-coding genes, these regions tend to be highly conserved across mammals, which does not fully explain the extent of phenotypic variation observed. This suggests that regulatory elements, particularly non-coding sequences, may play a more significant role in driving evolutionary change.

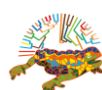
MicroRNAs (miRNAs) are short non-coding RNAs that regulate gene expression post-transcriptionally. Although there is substantial evidence to implicate microRNAs in organismal complexity, their biological relevance in mammalian evolution remains largely unclear due to limited annotation outside a few model species. Current miRNA prediction methods are heavily dependent on RNA-seq data, which is inconsistently available across mammalian taxa.

Materials and Methods: To address these limitations, this study developed a mapping-based approach to identify miRNA genes using homology, and explore their evolution across mammals. Using a curated set of known miRNA sequences as reference, orthologues were identified across 73 mammalian genomes. Candidate miRNAs were validated through a combination of synteny analysis and secondary structure assessment, and patterns of copy number

variation and gene family gain and loss were analysed in a phylogenetic framework. This homology-based method was then benchmarked against other currently used miRNA prediction pipelines.

Results: Using this approach, 3,519 orthologous miRNA families were identified across a broad range of mammalian genomes. Notably, 3,491 of these families were detected in species where they had not been previously annotated, demonstrating the method's capacity to expand miRNA repertoires in understudied species. Preliminary analysis suggests that patterns of copy number variation and gene family gain and loss may offer valuable insights into the evolutionary dynamics of miRNAs across mammals. When benchmarked against other commonly used annotation methods, this approach consistently identified more miRNA families across genomes.

Conclusions: Leveraging homology to identify miRNAs offers a powerful alternative to RNA-seq-dependent miRNA annotation and enables us to explore the complex evolutionary dynamics of these genes. These findings substantially expand the known miRNA repertoire in mammals and provide a rich resource for downstream evolutionary and functional studies. Overall, this work highlights the importance of studying non-coding regions to better understand genome evolution and its contribution to organismal complexity.



How does inbreeding affect local adaptation in the mountain plant *Arabis alpina*?

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POSTER SESSION 1 - P01.265, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The process of adaptation to the environment is impacted by many factors, one of which is the species' mating system. Self-fertilisation is a mating system occurring in 42% of angiosperms including rare species and crops. On one hand selfing causes extreme inbreeding and reduces the efficacy of selection, however theory also indicates that it may increase the fixation of recessive beneficial mutations and promote local adaptation by limiting pollinator-mediated gene flow. To better understand how inbreeding affects adaptation in nature, this project uses the wild mountain plant *Arabis*

alpina to test how intraspecific variation in the mating system has affected environmental adaptation. We have generated whole genome sequence and phenotypic data from replicate high and low elevation populations that are either self-compatible or self-incompatible. We will combine our new dataset with existing data and compare genomic signals of local adaptation to elevation between regions with different selfing rates. These data will help us understand how selfers differ from outcrossers in their response and resilience to environmental changes.



Mitochondrial Insights into the Unique Evolutionary History of Eastern Baltic Cod

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POSTER SESSION 1 - **P01.276**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The characteristic salinity and oxygen conditions of the Baltic sea have driven local adaptation processes in the Eastern Baltic cod (EBC), particularly in traits related to egg buoyancy and survival. Consequently, divergent selection on reproductive traits has emerged as a barrier to gene flow - even between EBC and the closely related Western Baltic cod (WBC). While divergences in the nuclear genome have been documented, it remains unclear whether similar patterns are mirrored in the mitochondrial genome. Mitochondrial DNA (mtDNA), due to its maternal inheritance, is a suitable marker for tracing the structure of female lineages in this population, where egg survival is linked to older, larger females. Additionally, given that mtDNA in Baltic cod is under neutral evolution, it offers high resolution insights into demographic history. Thus, this study aimed to assess whether local adaptation to the Baltic Sea was accompanied by the presence of one or a few dominant mitochondrial lineages, or whether it occurred within a background of multiple coexisting lineages. We analyzed 115 complete mitochondrial genomes from adult EBC individuals, alongside

comparative sequences from WBC and North Sea cod (NSC). Based on SNP data, we evaluated genetic diversity, population structure, demographic history, and haplotype relationships. EBC showed high genetic diversity, albeit slightly lower than NSC, yet encompassing nearly the full haplotypic diversity of the neighboring populations. Haplotype network revealed centrally placed NSC haplotypes with no shared nodes between NSC and Baltic individuals, suggesting a shared ancestry but no recent genetic exchange. The Baltic cod population originated around 10,000 years ago, during the postglacial expansion of the Atlantic cod into the newly forming Baltic Sea. These findings indicate that EBC harbors a historical mitochondrial diversity shaped by early colonization events, with few mutational changes since then. This suggests that the divergence seen in the nuclear genome observed nowadays is layered upon a much older maternal lineage structure. In this context, EBC is not only a product of recent local adaptation but also an important reservoir of deep evolutionary history, contributing uniquely to the overall genetic diversity of north Atlantic cod.



Narrow admixture zone between populations of the fungus *Trichaptum abietinum* in Norway

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¹University of Oslo

POSTER SESSION 1 - **P01.294**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

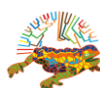
Glaciation cycles have shaped the genetic structure of species we see today. During glaciation species were pushed back into glacial refugia due to colder climate and ice sheet expansion. After glaciation, diverged populations could expand from refugia and come into secondary contact. Plants and animals in Europe show a common pattern in glacial refugia and postglacial migration, but it is unclear whether fungi follow the same pattern.

Trichaptum abietinum, also known as the purplepore bracket fungus, is a common white rot fungus growing on dead conifer wood. In Norway it grows on *Picea abies* and *Pinus sylvestris*. Recently two genetic groups of *T. abietinum* were discovered in Norway, the Atlantic group in western Norway and the Boreal group in eastern Norway. The two groups were suggested to result from two separate migration routes after the last glaciation. In the initial analysis, no admixed individuals were found. In Norway there are

strong climate gradients from west to east. Common garden experiments have indicated that local adaptation to climate may shape the current distribution of these populations.

We ask whether we will find an admixture zone in Norway when including a broader sample and if local adaptation to climate can be identified in the genomes.

We collected 40 samples throughout southern Norway, following the strong climate gradients. Full genome sequencing and population genomics tools have been used to investigate the research questions. We found an admixture zone in the innermost fjords west of the mountains. The admixture zone was confirmed with linkage disequilibrium decay and local ancestry analyses. Redundancy analysis and sliding window analysis of differentiation were also used to screen for candidate loci involved in local adaptation.



Of resilience, population growth, and genetic diversity

Scotti I¹, Boivin T¹

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POSTER SESSION 1 - P01.298, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

We have developed a simple model that combines population growth, genetic diversity, and resilience to disturbances.

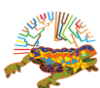
By introducing heritable variability in growth rates and sensitivity to disturbances, we show that genetic diversity interacts non linearly with the frequency of disturbances, pushing the population away from the equilibrium frequencies that can be predicted based on theoretical differences in fitness among genotypes.

Depending on the genetic covariance of survival and growth traits and on their genetic architecture, the interactions between disturbance frequency and genetic diversity generate varying long-term population growth rates.

This theoretical model shows that population resilience can be interpreted as a genetic property of populations and that disturbances can maintain genetic diversity in non-equilibrium systems, somehow recalling the intermediate disturbance hypothesis (IDH), applied at the genetic-diversity level.

We finally posit that the parameters of the model can be validated using field data to measure genotype-level growth and mortality traits. This will further allow us to predict the long-term fate of genetic diversity and resilience under climate change.

The model is generic, can be made arbitrarily complex, and can be applied to any biological system.



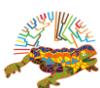
Phylogeographic and genomic insights unveil the evolutionary history and post-glacial recolonization routes of the Palmate Newt (*Lissotriton helveticus*) into Europe

Carranza B^{1,2}, López-Caro J^{2,3}, Jordà A^{2,3}, Spilani L^{2,4}, Talavera A², Mochales G², Cabana M⁵, Montori A³, Crochet P⁶, Recuero E⁷, García-París M⁷, Martínez-Solano Í⁷, Fernández-Guiberteau D³, Carranza S¹
¹Museu De Ciències Naturals De Barcelona, ²Institute of Evolutionary Biology (CSIC-UPF), ³Centre de Recerca i Educació Ambiental de Calafell (CREAC-GREN- Ajuntament de Calafell), ⁴Facultat de Biologia, Universitat de Barcelona, ⁵Grupo de Investigación en Biología Evolutiva (GIBE), Departamento de Biología, Facultad de Ciencias, Universidade da Coruña, Campus da Zapateira s/n, ⁶CEFE, CNRS, Univ Montpellier, EPHE, IRD, ⁷Museo Nacional de Ciencias Naturales, MNCN-CSIC

POSTER SESSION 1 - P01.273, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Quaternary glacial cycles have been key drivers of diversification for Holarctic species, promoting divergence, isolation, and extinction processes in numerous taxa. These cycles facilitated evolutionary radiations in some groups but also erased much of the evolutionary history of species with northern origins. Here, we investigate the evolutionary and phylogeographic history of the Palmate Newt (*Lissotriton helveticus*), a widespread species in post-glacial ecosystems in Western Europe. We generate genome-wide ddRADseq for 205 individuals from 51 populations across the species range and reconstruct its phylogeographic and demographic history, assess population structure, and characterize ecological paleo-niches for the species at different climatic periods. Results identify several distinct lineages exhibiting strong genetic differentiation, primarily driven by geographic barriers and isolation in historical

refugia with admixture in transition zones. Phylogeographic reconstructions suggest that the main glacial refugium for *L. helveticus* was most likely located in northern Iberia. Two main dispersal routes were identified: one extending eastward through the Ebro River Basin and, a second, following a north-eastward pathway across the Pyrenees and into Europe. We specifically pinpoint the origin of Europe's recolonization route to a specific set of localities surrounding Andorra, where *L. helveticus* probably expanded along tributaries to the Garonne River into southern and western France over warm periods. By integrating genomic, geographic, and paleoclimatic data, this study provides an in-depth understanding of how climate shaped the evolutionary history of this temperate species and reinforces the importance of waterways for amphibian dispersal dynamics.



'Plasticity of mutation rates and spectra in the green algae *Ostreococcus tauri* - Effects of temperature and salinity'

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POSTER SESSION 1 - **P01.274**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Phytoplankton forms an essential group of organisms, responsible for an estimated 50% of primary production with a major role in aquatic trophic networks and biogeochemical cycles. As oceans are changing rapidly, phytoplankton populations face intense selective pressures. To understand how these species will adapt to these changes, we must first understand how environmental conditions themselves influence genome evolution.

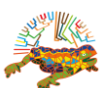
The ultimate source of genetic diversity upon which selection and genetic drift can act is formed by spontaneous mutations, which opens the door to evolution. Thus, the spontaneous mutation rate (μ), that is, the number of de novo mutations per generation per nucleotide, defines the adaptive potential of a species or population. Traditionally, μ is often treated as species-specific and stable; however, with advances in sequencing technology it has become clear that the environment has a major influence on μ .

We evaluated the plasticity of μ in *Ostreococcus tauri*, a marine picoeukaryote and model phytoplankton species. We performed mutation accumulation (MA) experiments under 4 different environmental conditions: high temperature (26°C), low

temperature (14°C), high salinity (50 g/L) and low salinity (20 g/L).

Short-read sequencing revealed that the base substitution rate (μ BS) doubled under high salinity and low temperature, and increased four-fold under high temperature compared to standard conditions. In addition, we sequenced a subset of MA lines with long-read PacBio and/or Nanopore sequencing to find structural mutations (SM) and estimate μ SM under the different conditions. This last technique also allows us to look at DNA methylation in the and make a correlation with changes in μ , taking another angle at the influence of epigenetic mechanisms on species adaptation. Finally, we use RNA-seq data allowed us to assess the influence of transcription on local mutation rates.

These results demonstrate that μ is highly plastic and changes with water temperature and salinity, 2 parameters that are expected to evolve significantly with global warming. This quantification of μ plasticity will be of service for studies on population dynamics and genetic diversity of phytoplankton species, helping to understand the influence of the environment on genetic diversity, which in itself is essential for resilience of populations against environmental changes.



Predicting adaptive potential from genomic data and its implications for conservation

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POSTER SESSION 1 - P01.260, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Adaptive potential, the capacity of a population to adapt, is essential to avoid extinction in a rapidly changing world. Therefore, accurately measuring a population's adaptive potential is crucial for informing effective conservation strategies. Molecular genetic diversity is an attractive and frequently used proxy of adaptive potential. However, previous research has cast doubt on the suitability of traditional molecular markers (allozymes and microsatellites) for predicting adaptive potential. In recent years, advances in

sequencing technology have instead shifted focus towards measuring nucleotide diversity and variation in functional regions. But the predictive power of these now favoured genetic measures is yet to be determined. Using literature-derived estimates from >80 species, we quantify the relationship between nucleotide diversity and the mean additive genetic variance of traits in a population– a theoretically well-motivated proxy for adaptive potential– to re-evaluate the usefulness of current genetic measures in a conservation context.



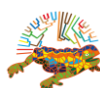
Predicting the genomic offset of fruit trees to climate change by building a reproducible bioinformatic pipeline

Criado M¹, Brisson M¹, Chen X¹, Venon A¹, Hansart A¹, Bourgeois Y³, Olivier F², Marchadier E¹, Cornille A⁴
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POSTER SESSION 1 - P01.264, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Climate change challenges biodiversity and food security, impacting organisms and ecosystems. Genetic diversity in crops and their wild relatives is vital for climate adaptation, as wild species historically supported crops adjusting to environmental changes. Therefore, investigating phenotypic plasticity and genetic adaptation of crops and their wild relatives is essential, though debates persist about their relative roles under climate change. We investigate the local adaptation and plasticity of four temperate perennial crops and their wild

relatives to climate change and predict their genomic offsets. We start with the apple tree as a model and will extend our analyses to other fruit trees. We present an unprecedented bioinformatic workflow that combines predictive tools, climatic data, and genomic variants that aim to be applied to a wide range of model systems and data. The genomic offset predictions are validated using climate-related fitness estimates recorded in common gardens planted in diverse European orchards.



Repeated local adaptation and genetic vulnerability to climate change in North American songbirds

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POSTER SESSION 1 - P01.299, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Across the Western Hemisphere, it has been estimated that half of all songbird populations are in decline. These declines are driven in part by disruptions to the genetic adaptations that enable these species to thrive in spatiotemporally heterogeneous environments. We have growing evidence that anthropogenic climate change is decoupling key genotype–environment associations, thereby accelerating population declines and local extinctions. At the Bird Genoscape Project (birdgenoscape.org), we are using next-generation sequencing methods to create maps of genetic variation - 'genoscapes' - across the breeding range of Western Hemisphere songbird species. Genoscapes are harnessed to track breeding populations to their respective wintering grounds, quantifying exposure to threats throughout the annual cycle. As part of this umbrella project, we are leveraging a comprehensive

whole-genome sequencing dataset comprising range-wide data for thousands of individuals in 39 North American bird species. Using this dataset, we aim to: (1) assess the repeatability of adaptation to climate by quantifying within-species associations between allele frequencies and climatic gradients; (2) evaluate how life-history strategies and ecological traits (e.g., migratory behaviour, range size, habitat) influence patterns of local adaptation; and (3) integrate our findings across species to produce comparative vulnerability maps that highlight populations and regions most at risk under future climate scenarios. By combining genomic, ecological, and spatial data, our research will provide novel insights into the genetic architecture of repeated local adaptation to climate in songbirds, and highlight regions and populations of conservation priority.



Selection mirage - Ecological selection led to repeated color changes during plant domestication

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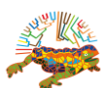
POSTER SESSION 1 - **P01.272**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Adaptation to environmental shifts often went along with numerous trait changes. This leads to the inference of selection on traits, but the connection between the novel trait variation and improved fitness often remains unknown. During domestication, plants adapted to agroecological environments and human preferences. During the process, a number of traits changed convergently across crop species in response to domestication and species have even been domesticated multiple times independently. Grain amaranth has been domesticated at least three times in different regions of the Americas. While some fitness-relevant domestication traits, including the loss of seed shattering and increased seed size, are not observed, the seed color has changed from dark to pale in all three crop species. The connection between seed color and environmental adaptation shows that selection 'sees' more than the human eye.

We identify the links between metabolic and gene expression networks controlling seed pigmentation through multi-omics. Domestication led to a strong downregulation of color pathway genes and a decline in pigment metabolites, leading to pale seeds. Genome-wide association revealed two major loci controlling seed color, of which one is causal for pale seeds.

The same locus was causal for seed color and germination differences in a mapping population. In an ecological assay, pale seeds were less dormant than dark seeds and their faster germination provided an advantage in agroecological environments without competition. After two years of storage, dark and pale seeds germinated equally fast, highlighting the contrasting adaptations to wild and agroecological environments. A haplotype network of the germination locus showed independent knock-out mutations in the same regulator gene as origin of pale seed color, highlighting the repeated emergence of pale seed color in the three domesticated grain amaranth species.

Pale seed color has emerged across numerous domesticated and wild species in response to human-mediated environmental change. We demonstrate how selection during environmental adaptation dragged along a seemingly fitness-unrelated trait through mediating pleiotropy of the regulator for germination properties. Our work shows that predicting the selective pressures from observed trait changes can be challenging and that interdisciplinary approaches, including ecology and omics are required to understand adaptation to future changes.



Selection pressures across the introgression landscape of Southeast Asian domestic cattle

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POSTER SESSION 1 - P01.280, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Introgression, or the movement of genomic regions between species, is prevalent in nature but its consequences are not well understood. It has been suggested that such interspecies gene flow could help promote adaptation to the local environment. Several domestic livestock breeds in Asia are known to have introgression from wild relatives which assist local adaptation to their environment alongside many other traits desired by humans. In areas of tropical climate, for example, *Bos indicus* is the more prevalent cattle breed as it is more resistant to pathogens and heat, unlike its relative *Bos taurus* that is more adapted to cooler climate in the northern part of the globe. However, it is not clear what controls which genomic regions are able to be introgressed, or why some background genomic variation persists. We used whole genome sequencing data of 90 individuals of admixed cattle from Indonesia (*Bos indicus* x *javanicus*, mean reads depth ~15x) coming from four different local breeds, i.e. Aceh, Pesisir, Pasundan, and Madura, to investigate the factors promoting introgression between species. From a previous study, these admixed Indonesian cattle were found to have experienced large

amounts of introgression from their wild relative, *Bos javanicus*, with differing proportions in different breeds. The abundant *Bos javanicus* ancestry encountered suggests positive selection towards these wild introgression, leaving questions on what impedes the other regions from receiving them and why different breeds have different proportions. To detect whether selection is among the factors shaping the persistence of *Bos indicus* genomic background in the admixed Indonesian cattle, we analysed genomes of these breeds that contain only the *Bos indicus* ancestry. We ran a number of selection scans detection as well as fitting demographic models to pinpoint the forces that shape their genomes. Additionally, we attempted to determine whether the genes appeared as being selected from the general selection scan were selected before or after introgression. We will discuss the forces that shape introgression landscapes in admixed cattle breeds, which will contribute to our more fundamental understanding of the genomic background of species barriers, a central question in the topic of speciation and therefore, the formation of biodiversity.



The Evolution of Plasticity and Heat Adaptation in Green Algae

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¹University Of Toronto

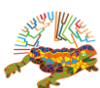
POSTER SESSION 1 - **P01.300**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Climate change significantly threatens biomes and their associated organisms and ecosystems worldwide, affecting species' functions and survival. The increase in temperatures and related environmental changes challenge the physiological limits of many species, potentially altering species' survival and interactions. The speed at which climate change occurs makes it difficult for species to adapt quickly, which can lead to accelerated decline and even extinction. Some species may respond to these changing conditions by relocating to more favourable environments, while others attempt to adapt through genetic changes or phenotypic plasticity. Phenotypic plasticity allows a single genotype to express multiple phenotypes, providing a potential mechanism for organisms to cope with climate change. However, the interplay between genetic changes and phenotypic plasticity is complex, as both contribute to an organism's phenotype and adaptation, and plasticity is often under partial genetic control. Thus, we aim to answer the following questions: How does plasticity influence adaptation to novel environments? Specifically, can long-term evolution evolve a plastic phenotype in response to fluctuating environmental temperature stress? And does plasticity aid or hinder the adaptation to more extreme temperatures? Here, we utilize the unicellular green algae *Chlamydomonas reinhardtii* to determine how organisms evolve and utilize phenotypic plasticity to adjust to temperature changes. The current body of research examines plasticity in response to acute heat exposure rather than long-term heat stress. Therefore, I aim to

understand the role of plasticity in the evolution of long-term heat responses in *C. reinhardtii* to extreme temperature changes. To first understand the extent of the natural variation in heat tolerance in various genetically distinct strains of *C. reinhardtii*, 10 natural strains were sampled from a latitudinal gradient from Quebec, Canada, to Florida, USA. Strains were exposed to temperatures ranging from 16°C to 39°C over four days, where their growth was monitored regularly via spectrophotometry. Despite differences in latitude, *C. reinhardtii* strains displayed similar fitness curves with an optimum growth temperature of 28-32°C and a sharp decrease in fitness after 32°C and ultimately cell death around 39°C.

A fluctuating temperature regime was used to evolve a plastic phenotype. Here, cell lines would fluctuate between the stressful environment of 20°C and 37°C every four to five days. Replicate cultures were grown for 9 months in either the fluctuating regime, a 20°C or a 37°C constant condition, and a 25°C control. Current data reveal that the fluctuating regime performs equally or better than its specialist counterparts, thus demonstrating that a plastic phenotype can adapt to extreme temperature stress via a fluctuating regime.

To understand the genetic regulation and mechanisms behind the evolution of heat tolerance and the plastic changes, cells used in the long-term evolution experiment will be sequenced to identify mutations of interest. Specifically, whether SNPs are shared between the fluctuating and constant growth regimes and if these SNPs foster heat tolerance



The Evolutionary Genomics Of Pesticide Resistance In The Water Flea *Daphnia Magna*

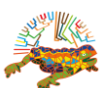
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¹KU Leuven

POSTER SESSION 1 - **P01.277**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

How does a changing environment shape the organisms' genome? The interplay between ecology, genetics, and development is a fascinating, yet complex, unanswered question within evolutionary biology. Despite the enormous advancements in molecular and computational methods, evolutionary studies combining genome sequencing and functional assays remain scarce at a landscape level. In the context of my PhD, I am using populations of the water flea (*Daphnia magna*) within an agricultural-vs-non-agricultural pond system in Belgium to study the evolution of their resistance to pesticides. The aim is a complementary approach to study their adaption, merging "traditional" population genetics tools with

state-of-the-art -omics technologies. Here, using 396 newly re-sequenced genomes, I will present the population structure of *D. magna* in this system, as well as the genomic loci that show signals of natural selection to pesticide resistance. By combining these results with gene expression and chromatin accessibility assays in the future, I aim to narrow down the loci that are functionally relevant to the protection against the pesticides, and identify coding and regulatory changes that are under selection. Overall, this study aims to provide a comprehensive insight into evolution in a landscape highly exposed to a prevalent anthropogenic stressor.



Time-Lagged Genetic Erosion from Habitat Fragmentation Undermines Evolutionary Resilience in Arthropods

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POSTER SESSION 1 - **P01.290**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

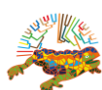
The intensification of land use over past millennia has dramatically accelerated habitat loss and fragmentation, processes that reduce population sizes and limit gene flow. These demographic shifts can amplify genetic drift, with potentially profound consequences for the evolutionary potential of species. However, empirical data on the population genomic impacts of habitat fragmentation—particularly in ecologically critical but presumed abundant taxa like insects and arthropods—remain limited. Increasing reports of insect and arthropod declines underscore their vulnerability, as many of these species have short generation times and sometimes low dispersal capacity, making them particularly sensitive to land use changes.

To assess how land use change and habitat fragmentation affects genetic composition and demographic trajectories, we combined historical agricultural land-use data from Denmark with whole-genome resequencing of 20–40 populations of each of 20+ arthropod species (beetles, collembola, spiders) from natural grasslands. Our results show that agricultural expansion has decreased habitat suitability, increased population isolation, and coincided with severe declines in effective population sizes. Gene flow estimates also revealed sharp recent reductions aligning with periods of agricultural intensification.

Notably, these changes in population size and connectivity have yet to fully manifest in

observed levels of genetic diversity due to a process known as “drift debt.” Following a population decline, genetic diversity does not immediately vanish; it erodes gradually over generations as a result of genetic drift. This time-lag means that populations may currently appear genetically healthy while still being on a trajectory of ongoing loss, particularly in the absence of gene flow. As a consequence, evolutionary potential is silently diminishing, with reduced capacity for adaptation to ongoing and future environmental stressors such as climate change.

We also perform environmental association studies and analyses of adaptive variation in response to environmental and climatic parameters, to identify how variation in life-history traits interact with environmental change to shape genomic structure and adaptive potential across arthropod species. Our findings highlight the potentially overlooked genetic risks facing even common species in fragmented habitats, and emphasizes the benefits of incorporating genomic analyses into management efforts to identify drivers of genetic erosion. Our study contributes to understanding how land use and environmental pressures shape genomic diversity, with important implications for the resilience and long-term persistence of biodiversity in a rapidly changing world.



Time-structured genomic sampling reveals abrupt and rare genetic shifts in *Eunicella singularis* populations, challenging snapshot connectivity estimates.

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POSTER SESSION 1 - P01.284, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Population connectivity (the exchange of individuals between populations) is a key process shaping metapopulation genetic structure, supporting population persistence and local adaptation. Therefore, accurately assessing connectivity is essential for effective ecosystem management, particularly in the context of spatial planning. It is often inferred empirically using genetic data, through approaches based on population genetic identity, genetic differentiation between populations, or the detection of first-generation migrants.

In coastal ecosystems, connectivity is primarily driven by the dispersal of pelagic larvae, and is thus influenced by ocean currents and species-specific life-history traits (e.g., spawning periods, larval duration). Both factors change over time, due to environmental and climatic variability. Yet, empirical connectivity studies typically rely on snapshot datasets, overlooking potential fluctuations through time.

Here, we investigated the robustness of connectivity assessments based on genomic data in a highly variable coastal environment. Using the temperate octocoral *Eunicella singularis* as a model species, we evaluated the temporal stability of population genetic identity, genetic differentiation, and inter-population recruitment patterns using a time-structured genomic dataset that include historical samples (2008–2014) and cohort-specific samples from 2022.

We show that large-scale genetic structure remains predominantly shaped by geography and that the genetic identity of most populations stays stable over time. However, some low-density populations exhibit marked

temporal shifts in genetic identity, likely driven by rare recruitment events involving genetically differentiated individuals. These shifts permanently alter the genetic identity of the affected populations and influence broader patterns of genetic differentiation. Furthermore, we identify rare inter-population migration events by detecting first-generation migrants, events that are unlikely to be captured by snapshot studies. Indeed, methods based on first-generation migrant detection may fail to identify such events if the relevant generation is not sampled, whereas estimates based on population genetic identity or differentiation may detect their consequences, but would not distinguish rare, massive migration events from low, stable migration patterns, thereby obscuring their episodic nature.

Our findings uncover the occurrence of rare, high-intensity connectivity events and emphasize their lasting impact on the genetic identity of *Eunicella singularis* populations, particularly in fragmented or declining populations. Unlike continuous low-level migration, these episodic events can drive abrupt genetic shifts, with profound implications for metapopulation dynamics and evolutionary trajectories. As such, they are likely to be underestimated or mischaracterized by conventional snapshot genetic studies. Recognizing and accounting for these infrequent but consequential events is essential for a more accurate understanding of population connectivity, especially in the context of accelerating population fragmentation and environmental change driven by climate change.



TO HAVE OR NOT TO HAVE: Does Having a Reference Genome Make a Difference?

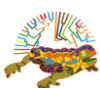
López Rivero A^{1,2}, Pascual M^{1,2}, Carreras C^{1,2}, Pegueroles C^{1,2,3}

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POSTER SESSION 1 - P01.278, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Conservation genomic studies in non-model organisms often rely on reduced representation sequencing methods with restriction enzymes, such as Genotyping-by-Sequencing (GBS), which can be employed without reference genomes. Understanding the benefits and limitations of reference genomes in population genomic studies is essential to evaluate their importance. To test the impact of having or not a focal reference genome in conservation genomics, we analyzed GBS data from two ecosystem engineers, the sea urchins *Paracentrotus lividus* and *Arbacia lixula*. We analysed their population structure and identify candidate loci using two approaches: a) de novo analysis without reference genome to infer individual genotypes for population structure and candidate loci identification, followed by mapping to *Strongylocentrotus purpuratus* genome and functional assignment, and b) mapping, genotyping, population structure and candidate loci identification using the recently published focal reference genomes. For the two approaches functional

characterization was carried out using EggNogg mapper. Both approaches yielded the same overall population structure, but the use of reference genomes resulted in greater differentiation between populations and lower inbreeding coefficients. In the first approach, less than 10% of loci mapped to the closest reference genome initially available (*S. purpuratus*). In the second approach, using their respective genomes $\approx 95\%$ of loci mapped. Both methods revealed a higher frequency of loci in exonic regions, consistent with the GC content of the restriction enzyme's recognition site. However, the functional identification of candidate loci did not improve significantly due to the limited knowledge in gene functioning in Echinodermata. These findings emphasize the reliability of de novo population genomic studies using GBS and they highlight the substantial improvement in genomic characterization with a reference genome but not their annotation, showcasing the limitations of reference genomes of non-model organisms.



Tracing genomic consequences of historical sealing in South American fur seal populations

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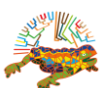
POSTER SESSION 1 - **P01.266**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Understanding the long-term genomic consequences of demographic history is central to evolutionary biology and conservation genomics. Demographic events such as population bottlenecks can leave lasting signatures in the genomic composition of populations - shaping genomic diversity, homozygosity, and accumulation of deleterious mutations. These signatures influence individuals' fitness and contribute to the adaptive potential of populations. Genomic data allow us to infer historical demographic events and evaluate how these past processes have shaped current patterns of genetic variation with implications for the future evolutionary trajectories of populations. Pinnipeds offer a 'natural experiment' for investigating how demographic histories leave lasting genomic signatures. Many pinniped species were heavily exploited by commercial sealers during the 18th and 19th centuries, resulting in bottlenecks of varying severity and duration. This variation makes them an ideal system to investigate these dynamics in a comparative framework.

Here, we focus on the South American fur seal (*Arctocephalus australis*), a species distributed across ~10,000 km of coastline along the Pacific and Atlantic coasts of South America. We compare Peruvian fur seals from the Punta San Juan marine protected area (Pacific coast) and Brazilian fur seals

from southern Brazil (Atlantic coast). While South American fur seals overall experienced moderate population declines due to sealing, historical records and genetic data suggest that the Peruvian population have undergone more severe and prolonged bottlenecks. In addition, the Peruvian fur seals is widely recognised as genetically distinct and has been suggested to be a separate evolutionary significant unit, likely shaped by both geographic barriers and historical demographic processes.

Using whole-genome resequencing data, we investigate how differences in demographic history affect genomic inbreeding, bottleneck strength, and genetic load. To do this, we estimate levels of inbreeding based on runs of homozygosity, reconstruct historical effective population sizes, and quantify putatively deleterious genetic variants. We expect to find evidence of a stronger bottleneck in the Peruvian population through demographic reconstruction. We hypothesise that the Peruvian population will show higher levels of genomic inbreeding and a higher burden of deleterious mutations compared to the Brazilian population. By quantifying and comparing these patterns, we aim to contribute to broaden our understanding of the long-term genomic consequences of historical demographic dynamics, and their potential impact on the fitness and resilience of wild populations.



Tracking Polygenic Adaptation: Phenotypic Stasis After Rapid Change in Large Populations of *Drosophila*

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POSTER SESSION 1 - P01.283, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Most quantitative traits are complex and their adaptation is driven by changes across many loci—polygenic adaptation. Most quantitative traits are under stabilizing selection which stabilizes the average trait mean on a specific value, i.e. trait optimum. Populations frequently experience environmental changes that shift the trait optimum, driving adaptation over short ecological timescales. Theory predicts that polygenic adaptation proceeds in two main phases: an initial directional phase characterized by shifts in the trait mean toward the new optimum, followed by a stabilizing phase in which the mean phenotype remains in equilibrium. However, the empirical data in support of the theoretical predictions is still lacking. We therefore aimed to empirically determine when adapting populations reached the new trait optimum after an environmental shift.

We quantified the phenotypic changes in replicate *Drosophila simulans* populations, each with 100,000 individuals, adapting to a new environment. An outbred founder population, created through round robin crosses of 93 inbred lines, was used to establish six large replicates that were exposed to a new high-protein diet for 31 generations in a laboratory environment. To track the evolutionary dynamics, we generated time-series high-order (fecundity, a fitness component) and molecular (gene expression) phenotypic data for the founder population (before the optimum shift) and the evolved replicates after 7 and 31 generations of maintenance on the high-protein diet. We characterized the trajectory of phenotypic adaptation and empirically inferred the transition between directional and stabilizing selection phases.

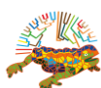
The fitness of evolving populations increased significantly at generation 7 (directional phase) then reached a plateau until generation 31. The directional phase was also characterized by a fast transcriptomic response at 7 generations involving more than 6000 differentially expressed genes (DEGs). After this rapid response, the expression of

a large proportion of the DEGs (64.73%, ‘plateau’ group) stabilized and reached a plateau until generation 31. The temporal patterns of gene expression are consistent with the patterns of fitness change suggesting the proximity of populations to the new trait optimum as early as generation 7. Patterns of parallel evolution indicated a high level of similarity among replicates where a high and consistent level of parallelism was observed in generations 7 and 31.

In addition to ‘plateau’ group other groups of DEGs with conserved patterns of co-expression were found. Two group of DEGs, ‘complete-reverse’ (11.17%) and ‘incomplete-reverse’ (5.30%) exhibited a reversal in the direction of expression change after generation 7 toward the ancestral population. Other group, ‘monotonic’ (7%) and ‘late-response’ (12.2%) showed a monotonous change in their expression until generation 31.

We computed the pleiotropic degrees of expressed genes using two independent measures: the tissue specificity and the level of connectivity in gene regulatory networks. Highly pleiotropic genes were less likely to be differentially expressed (i.e. constrained). However, groups of differentially expressed genes with transient responses (‘complete-reverse’ and ‘incomplete-reverse’ groups) exhibited the highest pleiotropy, suggesting the contribution of highly pleiotropic genes with large effect size in the initial phase of adaptation.

Here, we empirically inferred the transition between directional and stabilizing selection phases in populations adapting after an environmental shift confirming the predictions of theoretical studies. Our work helps bridge the gap between theoretical predictions and empirical evidence of polygenic adaptation over microevolutionary timescales. By combining experimental evolution with time-series phenotyping, we provide a framework to test and refine hypotheses about the mechanisms of adaptation.



Uncovering adaptive traits in legumes through novel genotyping approaches

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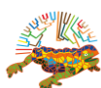
POSTER SESSION 1 - P01.263, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: The transition to plant-based diets is a crucial step toward ensuring sustainability of our agriculture and healthier dietary habits while contrasting and mitigating the impact of climate change. Legumes, due to their high protein content and ability to establish symbiotic nitrogen-fixing associations, represent a key resource for sustainable agriculture. Despite these advantages, genomic studies on food legumes are limited, which hinders breeding programs designed to promote their widespread use in agriculture. This is primarily due to the highly repetitive nature of plant species' genomes, which poses a major constraint on the large-scale sequencing of genotypes stored in germplasm banks, thereby limiting the comprehensive investigation of the biodiversity preserved across their numerous accessions. In this scenario, the GENUINE (Harnessing advanced Genomic mEthods to ideNtify fUture cllmate resilient geNotypes in lEgumes species with large and repetitive genomes) project aims to enhance the use of crop legumes as a natural resource for sustainable agriculture through the development of an innovative and cost-efficient genotyping platform.

Method: Specifically, the project focuses on: (i) Establish a sequencing pipeline based on Cas9 repeat-depletion system, designed to selectively remove highly repetitive, less informative genomic regions, (ii) applying this

approach to diverse genetic panels covering various agro-environmental regions, (iii) performing landscape genomics analyses to assess the impact of environmental and demographic factors on genetic variation, and (iv) integrating these findings with genome-wide association studies (GWAS) to identify genomic regions linked to both environmental adaptation and agronomic traits. The study will focus on lentil (*Lens culinaris*; ~3.69 Gb), pea (*Pisum sativum*; ~4.45 Gb), and faba bean (*Vicia faba*; ~13 Gb), three legumes of high nutritional and economic value that, given their prominent repetitive genomes (79% in faba bean, 84% in pea and lentil), will benefit from this technology. To this end, for each species a pool of accessions was selected to encompass their geographical diversity and, using passport data, we derived several environmental parameters which are crucial for landscape genomics experiments.

Results and Conclusions: Indeed, once the sequencing data were obtained, different statistical methods were implemented to correlate climate variables to genetic diversity identifying molecular traits linked with adaptation. Thanks to these results, it will be possible to guide breeding programs essential for improving legume cultivation, moving towards more sustainable agriculture and nutrition, and facilitating the adaptation of these species to future climate conditions driven by global warming.



Uncovering the genetic response of *Oikopleura dioica* against climate change-related biotoxins: insights into evolutionary enhance resilience

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POSTER SESSION 1 - P01.289, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

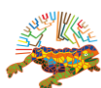
Background: The impact of climate change on the evolution of genomes is not only important to understand how it affects biodiversity resilience from an evolutionary point of view, but also from an ecological perspective aiming to mitigate its impact. Particularly in marine environments, higher temperatures are causing the increase in harmful algae blooms, releasing natural toxins such as polyunsaturated aldehydes (PUAs) that impair animal fitness, fertilization and embryonic development. Nonetheless, we have shown that a cosmopolitan zooplanktonic tunicate, *Oikopleura dioica*, have evolved resilience to these toxins, being affected in a less severe manner. The unique characteristics of this fast-evolving chordate—such as genome plasticity and extensive gene losses—make it an attractive animal model to study, not only conserved compromised developmental pathways, but more importantly, potential evolutionary adaptations underlying its resilience.

Materials and methods: We performed RNA sequencing on control and PUA-treated embryos of *O. dioica* on different developmental stages, from pre-zygotic transcription activation (ZTA) stage to mid-embryonic developmental stage. The obtained data was used to perform differential expression analyses and Gene Ontology (GO) enrichment analyses to identify affected pathways. Finally, phylogenetic analyses were performed on candidate genes to explore their evolutionary conservation.

Results: We identified several differentially expressed genes (DEGs) on each one of the

embryonic stages studied, highlighting potential early PUA-response pathways. Surprisingly, a high proportion of DEGs (from 40% to 58%) lacked GO annotations, suggesting the involvement of apparent many lineage-specific genes. Nevertheless, GO enrichment analyses showed the glutamine-glutamate pathway together with the energy metabolism as the top enriched categories, both conserved pathways needed for essential physiological functions. Furthermore, *O. dioica* was found to have duplicated a central gene of the glutamine-glutamate pathway: *glutaminase (GLS)*, showing four gene copies, all of them up-regulated in response to PUAs. Phylogenetic analysis suggested these duplications are lineage-specific, potentially contributing to the species' enhanced resilience.

Conclusions: Our findings indicate that *O. dioica*'s genetic response to PUAs involves both conserved and potentially lineage-specific genes. The expansion and up-regulation of *GLS* genes point to an evolved molecular adaptation enhancing glutamine–glutamate pathway activity under biotoxin stress. Additionally, the high proportion of DEGs lacking functional annotation raises the possibility of a novel marine toxin-response network. Together, these findings provide a foundation for understanding the impact of climate change-related stressors on marine animals and offer insights into both conserved and lineage-specific evolutionary adaptations contributing to environmental resilience.



Using admixture to understand local adaptation in the common wood decay fungus *Trichaptum abietinum*

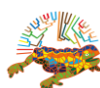
Lu D¹, Castellanos M¹, Andersen A¹, Helleland K¹, Solvik K¹, Methlie I¹, Kauserud H¹, Skrede I¹

¹University Of Oslo

POSTER SESSION 1 - **P01.297**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Trichaptum abietinum is a common wood decay fungus with a distribution that covers multiple climate zones. The widespread distribution of *T. abietinum* combined with its small genome size and ease of laboratory cultivation has sparked a growing number of population genomic and common garden studies. Previous studies have detected three genetic groups in Europe that likely originate from different glacial refugia. A Mediterranean group is found in Southern Europe whereas a Boreal group is distributed throughout Eastern Europe and most of Fennoscandia. These two groups form an admixture zone in central Europe where all the admixed individuals sampled to date have inherited the same large portion of a chromosome exclusively from the Boreal group. Wood decay rate experiments suggest heterosis in these admixed individuals in Central Europe as they have a faster decay rate than either parent population. A third Atlantic group has only been found in

Northwestern France and Western Norway. Fine scale sampling has revealed a narrow admixture zone between the Atlantic and Boreal groups in Norway. Interestingly, common garden experiments indicate that samples in Norway are better adapted to local conditions. The genetic regions involved these in adaptations are confounded with overall genetic structure since the distribution of the Atlantic group in Norway largely coincides with wet and mild climate, but the admixed individuals offer a way to control for this. Additional growth experiments with lab generated hybrids between the Atlantic and Boreal groups indicate that they are intermediates between the parent groups. We combine genetic, phenotypic, and bioclimatic data to identify genetic regions associated with local adaptation and try to understand these in light of the genomic landscape of introgression and recombination rate.



A locally adapted neotropical bromeliad shows loss of macrosynteny and expansion of stress related gene families among individuals along an elevational gradient

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MEETING ROOM 114, August 19, 2025, 11:00 AM - 1:00 PM

Local adaptation, a complex process driven by divergent natural selection, can involve both punctual mutations and extensive genomic rearrangements, affecting multiple metabolic pathways. Populations of the diploid bromeliad *Pitcairnia flammea* Lindl. display distinct phenotypic traits associated with ecological strategies along the elevation gradient of the Brazilian Atlantic Forest (from zero to ~2200 meters altitude). Our research aims to elucidate the genomic basis of local adaptation in *Pitcairnia flammea* Lindl. To achieve this, we assembled chromosome-level genomes of two individuals from contrasting altitudes (sea level vs. mountain) using PacBio HiFi and Omni-C sequences, and resequenced individuals with high-coverage Illumina short-reads from eight populations along an elevational gradient. The assembled genomes (1C = 500 Mbp) are highly contiguous, obtaining over 98% of Liliopsida BUSCO genes, but presenting large inversions in four chromosomes, encompassing ~170 genes in each genome. Both genomes are mainly composed of transposable elements (TEs), with five TE lineages particularly abundant in both genomes (i.e. Tekay, LARDs, SIRE, MITEs, TR_GAG), but showing variations between them. The PSMC analysis revealed that both individuals exhibited similar demographic histories, characterized by two population bottlenecks: one during the late Miocene (10 Mya) and the second during the Pliocene (1 Mya). The latter bottleneck coincides with an expansion of the LTR-TEs elements in both genomes. We detected an expansion of gene families in the mountain individual enriched

for GO terms related to water response, cold response, and lipid metabolism impacting several metabolic pathways, including the glycerophospholipid metabolism, which was previously reported to be involved in the adaptation to the temperature along the gradient in *P. flammea*. Moreover, the expanded gene families with these GO terms were duplicated and dispersed in the genome, with a higher density of the five previously identified TE lineages in their upstream and downstream regions compared to the rest of the genome. Thus, these results indicate that demography history or environmental pressure during the pliocene might have influenced the expansion of TE lineages. This expansion of TE might have played a role in expanding stress-related gene families, possibly mediating *P. flammea*'s adaptation to varying local environments along the elevational gradient. Notwithstanding, these results suggest that local adaptation in *P. flammea* populations might be driven by a complex interplay of multiple genes, diverse pathways, and changes in their genome architecture. The 81 individuals resequenced will be used to investigate the role of Single Nucleotide Polymorphisms (SNPs) and Structural Variations (SVs) in the adaptation process of *P. flammea*'s populations. Altogether, our study will contribute to a deeper understanding of the resilience of tropical mountain species to climate change, particularly in the world's most biodiverse region.



Adaptation to Multiple Environmental Stressors in *Tetrahymena thermophila*

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MEETING ROOM 114, August 18, 2025, 2:00 PM - 5:00 PM

Understanding how natural populations adapt to simultaneous environmental stressors is essential in the context of global change, yet most evolutionary genomics studies focus on isolated stressors. This limits our ability to predict population responses to the complex, multifactorial pressures characteristic of anthropogenically altered ecosystems. To address this gap, we conducted a long-term experimental evolution study using the ciliate *Tetrahymena thermophila*, evolving six clonal lines for ~1200 asexual generations under elevated temperature, chemical pollution, or their combination—two common stressors intensified by climate change. We measured adaptation as fitness changes in the evolutionary environment and applied an evolve-and-resequence approach to identify and track de novo single

nucleotide polymorphisms (SNPs). Contrary to expectations, the number of de novo SNPs did not differ across treatments. However, fitness gains were marginally but positively associated with the mean frequency of these variants, regardless of stressor type or number. These results suggest that adaptation under complex environmental conditions can proceed without an increase in mutational input, potentially via subtle shifts in allele frequencies. Ongoing annotation of the *T. thermophila* genome will aid in linking these variants to functional pathways involved in stress response. Our findings underscore the importance of integrating experimental evolution with genomics to better understand polygenic adaptation and resilience in the face of climate-driven environmental change.



Adaptive introgression enhances heat tolerance despite barriers to gene flow: a case study in *Drosophila flavomontana* and *Drosophila montana*

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MEETING ROOM 114, August 18, 2025, 2:00 PM - 5:00 PM

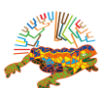
As global temperatures rise and heat waves become more frequent, populations must rapidly adapt to avoid local extinctions. Traditional adaptation through natural selection on new mutations or existing genetic variation has limitations. Hybridization and interspecific gene flow (introgression) offer an alternative route, allowing species to acquire pre-tested genetic material from close relatives. Few studies have experimentally tested the potential of adaptive introgression in response to warming temperatures, and investigated the interplay between adaptive introgression, barrier loci (regions of reduced gene flow), and recombination-suppressing chromosomal inversions.

Drosophila flavomontana's distribution is predicted to shrink due to increasing temperatures and the sterilizing effect of high temperatures on males. In contrast, *Drosophila montana* is more heat-tolerant, and therefore introgression from *D. montana* could aid *D. flavomontana* in adapting to heat. Despite diverging 2.5 million years ago, and possessing alternatively fixed inversions acting as potential drivers of speciation, the species can interbreed in the laboratory, making them ideal for studying gene flow and adaptation. We conducted a hybridize-evolve-and-re-sequence experiment to investigate whether heat exposure can facilitate the introgression of *D. montana* heat-tolerance alleles into *D. flavomontana* and improve heat tolerance. We also mapped long-term barriers between natural populations using a demographically explicit scan (gIMble) to explore whether

contemporary barriers observed in hybrids have also inhibited introgression throughout species divergence.

Heat-selected lines showed both improved male fertility under heat stress and greater introgression from *D. montana* compared to control lines. The X chromosome, with three overlapping inversions, showed no introgression in either heat-selected or control lines, indicating strong barriers to gene flow. In contrast, inversions on chromosomes 4 and 5 showed no introgression in control lines but some in heat-selected lines. The strongest correlation between male fertility and the amount of introgression was found in non-inverted regions of chromosome 4. Long-term barriers were also enriched within inversions and significantly overlapped with contemporary barriers.

Our results suggest that contemporary barriers observed in hybrids have acted as long-term barriers to introgression throughout species divergence, particularly within inversions. However, strong heat selection can overcome some of these barriers, especially in non-inverted regions and to some extent within autosomal inversions, facilitating the transfer of heat-adapted alleles between species and enhancing heat tolerance. While introgression has likely induced adaptation to warm temperatures, the levels of introgression were relatively low, and existing barriers appear to be strong enough to maintain species integrity.



Can Mediterranean Gorgonian Populations Cope with Extreme Climatic Events? Insights from Common Garden Experiments and Population Genomics in *Paramuricea clavata*.

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MEETING ROOM 114, August 19, 2025, 11:00 AM - 1:00 PM

Extreme climatic events (ECEs), such as marine heatwaves (MHWs), represent a significant and growing threat to marine biodiversity. Understanding the variability in ecological responses to recurrent ECEs within species, and the underlying drivers of this variability, is a key issue given its implications for conservation strategies and population recovery.

The red gorgonian, *Paramuricea clavata*, is a long-lived, habitat-forming octocoral dominating Mediterranean biogenic reefs. Shallow populations of this species have suffered mass mortality events due to recurrent MHWs over recent decades. Notably, field observations reveal variable mortality rates across populations, suggesting differing levels of thermal vulnerability and the potential for identifying both “resistant” and “sensitive” individuals—an insight that opens promising avenues for climate-resilient restoration efforts.

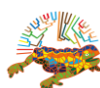
Here, we present the results of a collaborative effort that combines experiments in controlled conditions, genomics and population genetics, to characterize the drivers involved in the differential vulnerability to thermal stress.

In the first experiment, we exposed samples from 11 Mediterranean populations to thermal stress under common garden conditions to characterize response

variability across a broad spatial scale. From this, we re-sequenced the whole genome of 50 “resistant” and 50 “sensitive” individuals, and conducted a genome-wide association study (GWAS) to examine their genomic backgrounds. The results indicate no shared major-effect loci linked to thermal resistance, suggesting a complex or polygenic basis for this trait.

In a second experiment, we conducted repeated thermal stress tests over three consecutive years (2015–2017) using the same individual colonies. Statistical modeling highlighted the summer thermal regime prior to each experiment as the main factor influencing the response of *P. clavata* to thermal stress. Adaptive mechanisms—whether genetic or related to phenotypic plasticity—appeared to play only a limited role.

Overall, our results indicate that *P. clavata* has a constrained adaptive capacity to cope with the increasing frequency and intensity of marine heatwaves in the Mediterranean. We discuss the implications of these findings for the sourcing of individuals in climate-resilient restoration efforts. These findings raise important questions about the viability and effectiveness of current conservation and restoration strategies, emphasizing the need to reassess sourcing practices and long-term resilience goals for *P. clavata* in a warming Mediterranean.



Contrasting contemporary changes in large-effect life history loci in *Salmo salar* across Europe

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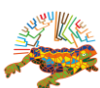
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Understanding how wild populations evolve in changing environments is a central aim in evolutionary biology. In the last few decades, Atlantic salmon (*Salmo salar*) populations have been facing human-induced declines, as well as shifts in age at maturity and growth across their distribution. However, it is unclear whether these changes reflect evolutionary adaptation and how important large-effect loci are in this regard. Age at maturity, the number of years an individual spends feeding at sea before maturation, is an important fitness trait in Atlantic salmon. The genetic architecture of this trait includes two large-effect loci, *vgll3* and *six6*, which explain a substantial fraction of the phenotypic variation. The availability of long temporal data combined with this simple genetic architecture makes this trait a model for understanding contemporary evolution. In this study, by genotyping thousands of samples from long-term data collections across multiple wild Atlantic salmon

populations, we track the dynamics of change in the age at maturity trait and associated large-effect loci throughout the European range. Our results provide evidence for range-wide rapid adaptive evolution of some large-effect life history loci, but the direction and magnitude of change differs among populations. For example, somewhat surprisingly, opposing directions of allele frequency changes were observed in large effect loci in different populations. These findings illustrate heterogeneous adaptive responses in wild Atlantic salmon along a broad distribution spanning 42 to 65 years. Such responses are likely driven by different ecological and environmental pressures, such as riverine and marine fishing, water temperatures or the abundance of key prey species.

Keywords: Age at maturity, adaptive evolution, natural selection, life history, *Salmo salar*



Cryptic diversity and phylogeographic patterns in Mediterranean acrobat ants (*Crematogaster*) reveal complex evolutionary histories in response to climate change

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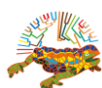
Understanding how insects have responded to past climate oscillations—through range shifts, migration, or adaptation—is crucial for refining future predictions and implementing effective conservation strategies. Yet, evolutionary histories of insects at the population level remain understudied. We address these critical questions by focusing on Mediterranean-wide distributed ants of the genus *Crematogaster*, one of the most species-rich and evolutionarily successful ant genera worldwide.

We investigated the *C. scutellaris* species complex, inhabiting ecologically dominant and behaviorally diverse members of Mediterranean ecosystems, playing key roles in shaping arthropod communities and influencing ecosystem dynamics. We generated low-coverage whole-genome sequencing (lcWGS) data for populations across their Mediterranean distribution. Genome-wide SNPs were analyzed to infer phylogenetic relationships, assess population structure, and detect cryptic diversity. BUSCO genes were used to evaluate genome completeness and support deeper phylogenetic signal. Genomic patterns were examined in regions of sympatry to explore introgression or hybridization events. In comparison, we analyzed populations of *C. sordidula*, another widespread Mediterranean ant species, using ultraconserved elements (UCEs) and SNPs to construct phylogenies, assess population structure, and estimate divergence times relative to historical habitat and climate changes.

Our analyses revealed deep genetic divergences within the *C. scutellaris*

complex, including a pronounced east–west split, a biogeographic pattern widely observed in Mediterranean taxa. Genome-wide data uncovered multiple genetically distinct clusters, suggesting extensive cryptic diversity undetectable through morphology alone. In zones of sympatry, particularly on the Balkan Peninsula, our data indicates historical and possibly ongoing hybridization. These findings point to dynamic population interactions in areas shaped by fluctuating environmental conditions. For *C. sordidula*, genomic data revealed comparable east–west differentiation, with divergence times corresponding to major climatic and geological events during the Pleistocene. The Aegean region and the Balkan Peninsula exhibited especially complex population dynamics, reflecting habitat complexity shaped by intense climatic oscillations. Southern Mediterranean refugia, which remained relatively stable during these climate shifts, emerged as critical centers for lineage persistence across species.

Our study reveals a complex evolutionary history in *Crematogaster* ants shaped by repeated climate and habitat changes. Genome-wide molecular data provide strong support for the molecular delineation of cryptic lineages and uncover a more intricate evolutionary history and complex population structures than previously inferred from morphology alone. These insights contribute to a deeper understanding of insect response to past habitat changes and inform predictions about their adaptability in the face of future climatic challenges.



Deep-time genomes reveal the evolution of distinct genetic variants in the woolly mammoth

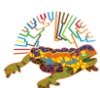
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The unique characteristics of a species' genome result from evolutionary changes accumulated across considerable timescales, often exceeding several hundreds of thousands of years. While comparative genomics allows for the identification of genome architecture changes across species, pinpointing the exact timing and rate of these evolutionary changes remains challenging when relying on modern snapshot data alone. The Pleistocene evolution of mammoths in Eurasia represents a framework for studying the evolutionary processes of environmental adaptation and speciation in changing climates. Here we sequenced 29 late-Pleistocene woolly mammoth genomes as well as two mid-Pleistocene mammoths, dated to 1.1 and 0.7 million years old. Combining this with 64 additional woolly

mammoth, African elephant, and Asian elephant genomes, we identified the protein-coding and regulatory region changes that underlie the formation and speciation of the woolly mammoth across a million-year time transect. We find that most functional changes for cold climates and high latitudes in woolly mammoths evolved in the time before the first woolly mammoths appear in the fossil record around 0.7 million years ago. Genes involved in fat metabolism, thermoregulation, and immune system functioning have been under continuous selection in woolly mammoths up to their extinction, whereas genes involved in fur and hair development, circadian rhythm, and taste and smell perception had already highly evolved in the mid-Pleistocene mammoths and remained stable since.



Effect of temperature on viability selection of developing hybrids revealed by whole genome pool-seq

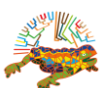
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Hybridization is now known to be pervasive across the tree of life and to have deleterious consequences, e.g. selection against hybrid incompatibilities. However, it can also allow for fast adaptation to changing environments via adaptive introgression or by increased genetic diversity that allows for faster adaptation. With higher temperatures and frequency of extreme events due to climate change, species ranges are predicted to shift and bring previously geographically isolated species or populations into contact, increasing hybridization rates. Further, recent research has proposed that hybrid plants and animals might have increased fitness under climate change. Thus, it is necessary to understand if hybridization in the context of climate change will be adaptive (i.e., allow admixed populations to better adapt to their fast changing environments) or deleterious (e.g., due to selection against hybrid incompatibilities). An outstanding system to study these questions can be found in Southern Finland, where the mound-building wood ants *Formica aquilonia* and *F. polyctena* have hybridized and produced several hybrid populations. Thermal stress experiments have shown that the parental species are divergently adapted to temperature (*F. polyctena* is warm-adapted, while *F. aquilonia* is cold-adapted) and that hybrids are as tolerant to heat as their warm-adapted parental species. In haploid

hybrid males, the frequency of cold-adapted alleles rises in colder years and that of warm-adapted alleles rises in warmer years. Hence, temperature is likely a key selective pressure to which hybrids can respond quickly. However, this increased thermal resistance was observed in adult workers; how are hybrid queens and their offspring affected by temperature? To address this, we exposed hybrid and cold-adapted parental queens to different temperatures and compared their egg-laying and hatching rates. We show that the higher thermal tolerances to different temperatures found in adult hybrid and parental workers are absent during early development. We found that low temperature is a severe selective pressure for both hybrids and parentals. The hatching rate of hybrid eggs was reduced compared to parental eggs at high temperature, which could be explained by environment-dependent hybrid incompatibilities. Ongoing analysis of pooled whole genome sequencing data obtained from eggs and larvae collected from this experiment will unravel signatures of intrinsic and environment-dependent incompatibilities, allowing us to quantify the strength of selection against incompatibilities across the genome. Overall, we show that developing individuals need to overcome the deleterious effects of hybridization during development to reap its benefits in adulthood.



Environmental effects on the origin and establishment of *Arabidopsis arenosa*

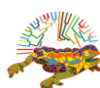
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The effect of the environment on the establishment of polyploidy has been a long-term mystery, and it has been hypothesized that polyploids are better adapted to temperature extremes than their diploid progenitors. Given the increasing challenges that plants and animals face due to global warming, this study explores polyploids' molecular basis of their adaptation to thermal and precipitation fluctuations, using *Arabidopsis arenosa* from the family Brassicaceae as the model system. This species crosses multiple climatic zones along its distribution, and its various intraspecific lineages adapted to diverse environmental conditions, including northern latitudes. In this study, I utilized the first pan-genome of *A. arenosa* and genotype-environment association analysis with 254 genome-sequenced diploid and 510 tetraploid populations (total 764 individuals) to investigate the impact of harsh environments on the adaptation of *A.*

arenosa, namely selected genes. Also, the genetic offset was used to predict the maladaptation comparing diploids with tetraploids. Based on the initial findings from this analysis, significant differences in single nucleotide polymorphisms strongly associated with the coldest temperature in the coldest month and precipitation of the driest month were observed between diploids and tetraploids. Our preliminary findings indicate that genes strongly associated with cold temperatures in tetraploid *A. arenosa* encompass both stress response and DNA regulation, while for precipitation genes involved in signaling receptor response to stress were found specifically in tetraploids. This may explain why tetraploids exhibit a robust adaptation to wider environmental regimes, although it is not significantly stronger compared to their diploid progenitors.



Evolutionary genomic predicts Adaptation, Maladaptation, and Plastic Expression Responses to Climate Change in Two Closely Related Species with Contrasting Distribution Ranges and Population Sizes

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Background: Climate change is transforming ecosystems globally, exerting selective pressures that threaten the survival of many species, including forest tree species that play crucial ecological roles in maintaining the structure and function of terrestrial ecosystems. The ability to adapt, avoid maladaptation, or exhibit plasticity in response to environmental changes is essential for species' persistence under such conditions. Evolutionary genomics provides a powerful framework to investigate these dynamics by identifying genetic variants associated with climate adaptation, quantifying genomic vulnerability and genetic load under future climate scenarios, and analyzing gene expression plasticity as an immediate response to stress.

Materials and methods: In this study, we focused on two closely related species with contrasting ecological and demographic characteristics: *Populus adenopoda*, a widespread subtropical mainland species inhabiting diverse environments across East Asia, and its sister species, *Populus qiongdaoensis*, a tropical island species restricted to Hainan Island with small population sizes. We generated high-depth whole-genome resequencing datasets for 228 individuals sampled from 32 populations of *P. adenopoda* and 5 populations of *P. qiongdaoensis*.

Using this dataset, we first reconstructed the phylogenetic relationships between the two species, inferred their divergence history, and analyzed the population structure of their current populations. Next, we applied various approaches to assess and compare future habitat suitability, the accumulation of deleterious mutation loads, and genomic offsets to future climate change across all populations within the distribution ranges of both species. This allowed us to evaluate the adaptive capacity and the ecological and genomic vulnerabilities of these populations and species. Finally, we assessed the extent of gene expression plasticity in response to immediate environmental stresses by performing greenhouse experiments. These experiments involved collecting gene expression profiles from both leaf

and root tissues of seedlings from both species under control and environmental stress conditions, including heat and waterlogging.

Results: First, our results revealed that *P. adenopoda* and *P. qiongdaoensis* diverged approximately 2.51 million years ago during the Quaternary glacial period. Following their divergence, *P. qiongdaoensis* experienced a continuous population decline, whereas *P. adenopoda* likely contracted into three major refugia and underwent substantial population expansion, eventually spreading across the subtropical mainland of East Asia after the Last Glacial Maximum, around 20,000 years ago.

Second, compared to the widespread species *P. adenopoda*, the island species *P. qiongdaoensis* exhibited significantly lower genetic diversity, a higher genetic load, and greater genomic vulnerability to future climate change. As the island species cannot migrate to more suitable habitats, assisted gene flow appears to be a promising strategy for mitigating maladaptation. Through an integrative approach, we identified optimal locations and adaptive genes within the range of *P. adenopoda* that could effectively transfer adaptive variants via assisted gene flow to support the survival and adaptation of *P. qiongdaoensis*.

Lastly, we examined and compared the expression plasticity induced by instant environmental stresses in seedlings of both species under greenhouse conditions. This analysis provided insights into the relative contributions of adaptive and non-adaptive plastic expression responses in facilitating adaptation to novel environments.

Conclusions: By integrating ecological, genomic, and transcriptomic data, our study explores how demographic history, genetic diversity, and environmental exposure shape adaptation and maladaptation in two sister *Populus* species with contrasting characteristics. These findings provide valuable insights for conservation strategies, including assisted gene flow and genetic rescue, to mitigate climate-driven risks for the island species.



Evolutionary trajectories of plasticity when encountering novel thermal conditions during range expansion in the wild

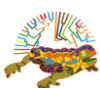
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Despite the theoretical importance of plasticity for the speed of adaptation to novel environments, empirical data on the evolutionary trajectory of plasticity in new selective regimes are very limited in the wild. We harnessed range expansions of a damselfly from France into warmer regions in Spain as a natural experiment to test how transcriptional thermal plasticity evolves, and compared the outcomes with a native, locally thermally adapted sister species. A common-garden experiment of 11 populations revealed that plasticity evolution depended on the magnitude and duration of the warming treatment, with

stressful high temperatures causing maintenance of plasticity. The frequency of plastic genes that becomes assimilated is higher with increasing duration of historical exposure to the thermal regime and when the warming treatment is more often encountered. Overall transcriptomic plasticity first increased and subsequently decreased during range expansion, indicating evolved plastic responses precede evolutionary adaptations when coping with new thermal environments. These findings are crucial to predict the role of plasticity in driving evolution in a warming world.



Genes that underlie photoreception and seasonal reproduction are crucial for local adaptation in the Northern krill, a keystone zooplankton for the North Atlantic Ocean

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Krill are superabundant pelagic crustaceans that play crucial roles in ocean food webs and influence global climate by acting as ocean carbon sinks (Trinh et al, 2023). Climate change is strongly impacting the distribution and well-being of krill populations (Veytia et al, 2020), but little is known about the adaptive potential of krill species to a changing environment. To date, genetic studies of krill have been hampered by their large genome sizes. In contrast to polar species like the Antarctic krill, the boreal Northern krill, *Meganyctiphanes norvegica*, is adapted to both warm and cold water, and therefore serves as a good model system for adaptation. To better understand processes of genetic adaptation in Northern krill, we performed de novo genome assembly and population genomics on 74 individuals from 8 populations distributed over the North Atlantic Ocean and the Mediterranean Sea (Unneberg et al., 2024).

We used multiple DNA sequencing technologies to generate a 19 Gbp reference genome assembly of a female specimen in the Gullmarsfjord, Sweden. The genome is highly repetitive and has evolved through wide-spread proliferation of retrotransposons, now targeted for inactivation by an active DNA methylation system. In addition, gene families for photoreception and molting are greatly expanded through gene duplication, which suggests adaptation to temperature and light.

We resequenced 74 individuals from 8 populations and compiled a dataset consisting of ~760 million SNPs. Genetic variation analyses revealed moderate levels of genetic diversity (~1.4% overall) and identified extensive homogenizing gene flow. Nevertheless, hundreds of genomic loci did show highly divergent allele frequencies between populations, likely due to selection for local adaptation. Candidate genes showing signs of adaptation were predominantly

involved in photoreception, circadian rhythm, reproduction and thermal tolerance. The top candidate gene was *nrf-6*, a gene that codes for a protein involved in ovary maturation and oogenesis. One haplotype is fixed in the Mediterranean population and could be a locally adapted reproductive behavior to early spring phytoplankton blooms.

With our work, we have established the Northern krill as a model for ecological, evolutionary, and population genomics in zooplankton. Our variation data and candidate gene list is a valuable resource for researchers who study the link between genetic variation and adaptation. A better understanding of genetic adaptations of krill will contribute to improved predictions of their long-term responses to environmental changes and provide guidance for stock management and conservation efforts.

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Genetic variation in the response to multiple stresses: the case of *Lymnaea stagnalis* exposed to metals and global warming

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Background: Climate change and pollution are among the five most important threats posed to biodiversity (Parmesan et al. 2022). Besides, it is now admitted that the unprecedented levels of environmental stress undergone by biodiversity lead to time scale overlaps between ecological and evolutionary responses (Carroll et al. 2007). As chemical pollutants constitute potent sources of environmental stress, their ability to trigger micro-evolutionary processes in exposed populations is increasingly studied. Understanding the evolutionary impact of these pollutants is also a new challenge for ecological risk assessment, especially in the context of global change where populations are often exposed to complex cocktails of contaminants along with other anthropogenic and natural stressors, which may interact in various ways. Therefore, it has become critical to assess chemicals' long-term ecological risk under various climatic scenarios.

A first step towards this end is to evaluate the genetic variability in stress responses, upon which selection might operate. In the present study, we addressed this issue by assessing the between-population variability in transcriptomic responses of a model invertebrate to trace metallic elements (as widespread persistent contaminants) and warming. We chose copper, as an essential element that becomes toxic at high doses, which is widespread and used as pesticide even in organic agriculture, and lithium, as an emergent contaminant associated with the development of digital technologies).

Materials and methods: To this end, seven day-old F1s obtained in the laboratory from adults collected in five populations or strains of the great pondsnail *Lymnaea stagnalis*, were exposed for three weeks (juvenile period) to a sublethal concentration of lithium (100 µg/L) and copper (20 µg/L), separately or as a mixture, under two temperature regimes (20°C, considered as optimal under local conditions,

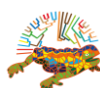
and 24°C, following a +4° warming scenario). The effects of metals and temperature were estimated on transcriptomic expression (differential expression relative to controls, RNAseq), together with snail growth and cardiac frequency, and compared across populations.

Results: Growth was faster at 24°C than at 20°C, reduced under copper exposure, and not affected by lithium. The two factors interacted significantly with the factor population, indicating genotype x environment interactions at the level of populations.

Cardiac activity was negatively related to age and size, and this relation was steeper at 24°C, especially under lithium and control conditions (Temperature by Metal interaction). Population had no effect alone nor interacted with other factors on this trait.

Transcriptomic analysis (Deseq2) showed a main effect of temperature, with 711 differentially expressed genes (DEGs). Among these, only 68 DEGs were shared by the five population. Lithium had almost no detectable effect on the transcriptome, whereas copper exposure induced substantial differential expression (538 DEGs), with no gene found in common among the five populations. While these results reflect a strong influence of the genetic background on the response of *L. stagnalis* to the tested factors, the functional significance of this between-population component of transcriptomic expression is currently explored through annotation (with a particular attention to regulatory factors) and enrichment analyses, which will be presented at the conference.

Conclusions: The present results suggest that a species ability to cope with thermal and metal stress, as well as their combination, depends strongly on the population genetic background. Such interactions indicate that current ecological risk assessment of chemicals, which ignore warming and genetic influences, may be underprotective.



Genomic bases of short term evolution in the wild revealed by long term monitoring and population-scale sequencing.

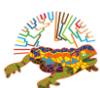
Cumer T^{1,2}, Topaloudis A^{1,2}, Lavanchy E^{1,2}, Ducrest A¹, Sarmiento Cabello S¹, Becciu P¹, Hewett A^{1,2}, Simon C¹, Almasi B³, Roulin A¹, Goudet J^{1,2}

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MEETING ROOM 114, August 18, 2025, 10:30 AM - 12:30 PM

Understanding how populations rapidly adapt to changing environments requires uncovering the genomic basis of heritable traits and examining how these traits evolve over short timescales. This challenge is particularly acute in wild, non-model species, where disentangling genetic and environmental contributions to phenotypic variation demands extensive phenotypic and genomic resources. The long-term study of the Swiss barn owl (*Tyto alba*) population provides a rare and powerful framework for addressing this challenge. Over the past 30 years, we have observed a striking evolutionary trend: individuals are becoming darker and more spotted—two melanin-related traits that were previously linked to fitness. Leveraging whole-genome

sequencing of over 3,000 individuals, we show that these two traits are controlled by a few loci with substantial effect sizes and exhibit a partially overlapping genomic architecture. Temporal allele frequency tracking at these loci shows subtle yet consistent changes. Using simulations, we show that these changes align with patterns expected under selection. By illuminating the genomic mechanisms underlying rapid phenotypic evolution in a wild population, this study provides critical insights into how fitness-related traits evolve in response to environmental pressures. In the context of accelerating climate change, our findings underscore the potential for adaptive responses in natural populations.



Genomic signals of adaptation during replicate range shifts of the blue-tailed damselfly, *Ischnura elegans*, in Scotland and Fennoscandia

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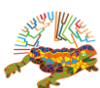
MEETING ROOM 114, August 18, 2025, 2:00 PM - 5:00 PM

With climate change, range shifts to higher latitudes are now widely observed across plants and animals. Recent work has shown that range-expanding populations can rapidly adapt to new environments, fundamentally changing the expansion velocity and final range limit. However, the genomic changes underlying range shifts in wild populations are still poorly understood. How variable and predictable are genomic changes during climate-mediated range shifts, and how do environmental gradients affect gene flow, drift, and adaptation?

We explored these questions along four replicate range-shifting transects of the blue-tailed damselfly, *Ischnura elegans*, in Scotland, Norway, Sweden, and Finland. This species is rapidly expanding its native range northward, consistent with insects showing especially rapid poleward movement in response to climate change. We conducted low-coverage whole genome sequencing of 957 individuals from 81 sites spanning 1700km. To study neutral and adaptive genomic changes along the transects, we analyzed the sequencing data using a genotype likelihood framework in ANGSD. We conducted a Genome-Environment

Association analysis across the genome for each transect separately, and then compared putatively adaptive SNPs across the transects in windows of 10 kbp using PicMin, a statistical tool designed to detect repeated adaptation in genome scans.

Population structure strongly differed between the transects, consistent with regional differences in dispersal barriers in the landscape. We found 61 genomic windows that showed significant association with the environment across all 4 transects, with temperature being more important than precipitation. Outlier windows revealed candidate loci with annotations relevant for poleward movement including the circadian clock, development and immune function. Using local PCA, we identified a putative structural variant that was more common in cold environments and associated with neuropeptide receptors involved in insect feeding, locomotor activity and circadian rhythms. By comparing parallel outlier loci with idiosyncratic outliers unique to individual transects, we aim to better understand the genomic drivers of adaptation during range shifts.



Local adaptation and forecasting response in the endangered Scottish freshwater pearl mussel

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MEETING ROOM 114, August 18, 2025, 2:00 PM - 5:00 PM

Given the rapid pace of climate change, there is an urgent need to understand how endangered species will respond to changes in their environment. Species distribution modelling is a popular tool used to forecast a species range based on the relationship between surveyed occurrence data and explanatory (habitat) factors. However, it does not account for local adaptation and intraspecific response to change. Genomic offset offers an evolutionary dimension for forecasting species response to change and is defined as the mismatch between current adaptive composition and future optimal composition under a changed environment. The freshwater pearl mussel, *Margaritifera margaritifera*, has a widespread Holarctic distribution but many constituent populations are endangered or threatened. Scottish populations have a complex pattern of genomic population structure, partially explained by local climate. Here, we compare predictions from an ensemble species distribution model (SDM) of 462 presence points of *M. margaritifera* with

genomic offset estimates derived from 417 putatively adaptive SNPs from 18 populations in Scotland. Both the SDM and genomic offset forecast a loss of *M. margaritifera* from the eastern Scottish Highlands, with loss of suitable habitat and maladaptation of local populations. However, while the SDM indicates a future loss of suitable habitat on the Ardnamurchan Peninsula, these populations have a lower value of genomic offset suggesting these populations are more resilient to climate change through existing adaptation. As such, these findings indicate that although general trends are congruent among methods, there are fine scale differences that may go undetected without considering genomic composition. This study examines the use of both species distribution models and genomic offset and demonstrates the utility of an evolutionary perspective alongside species distribution models for forecasting species response to a changing climate.



Local Adaptation to Climate in Wild and Feral Brassica rapa: Genetic Architecture and Genetic Offset

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MEETING ROOM 114, August 19, 2025, 11:00 AM - 1:00 PM

As climate change drives rapid environmental shifts, crops face significant challenges due to their limited genetic diversity, which constrains their adaptive potential. Crop wild relatives—and potentially feral populations, which are wild populations derived from domesticated ones—are likely to be locally adapted to diverse environments and could serve as valuable reservoirs of genetic diversity for breeding and crop improvement. Exploring and conserving this genetic diversity is essential, as it preserves a broad spectrum of adaptations to varied environmental conditions.

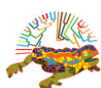
In this study, we investigate the genetic basis of local adaptation to climate and potential mismatches under future conditions in *Brassica rapa* (field mustard), a wild relative of turnip, Chinese cabbage, and pak choi, and a genome donor for *Brassica napus* (rapeseed). Seeds were collected through the BrasExplor project—a consortium coordinated by IGEPP (INRAE) with 11 partners from seven countries. A total of 62 spontaneous populations were sampled in Algeria, France, Italy, and Slovenia, revealing strong genetic structure between feral populations (from continental Europe) and wild populations (from Algeria and Sicily).

We conducted genome–environment association analyses and applied the local score approach to identify highly significant genomic regions associated with climatic variables, including promising variants and candidate genes. However, broader genomic patterns suggest that adaptation to climate is not limited to a few loci but likely reflects a

polygenic architecture involving multiple regions across the genome. While the inclusion of feral populations allows exploration of a broader climatic envelope and identifies variants potentially easier to introgress into crops, their reduced genetic diversity—likely due to domestication and rewilding bottlenecks—may limit our ability to capture the full spectrum of adaptive variation present in truly wild populations. This hypothesis was confirmed by repeating the analysis on a subset of wild populations, revealing a large number of additional candidate regions.

We also conducted genomic offset (GO) analyses to estimate the potential mismatch between current allele frequencies and those expected under future climates. Several populations showed high GO values, suggesting nonoptimal allele frequencies in their future climate. Phenotypic data obtained in a common garden experiment indicated that GO may partially predict variation in fitness, but this relationship should be interpreted with caution. More specifically, fitness proxies (seed number and weight) may be biased in feral populations, and much of the adaptive signal specific to wild populations is not captured, limiting predictive power for those populations.

These findings highlight the complex genetic architecture of climate adaptation in *B. rapa* and underscore both the potential and the limitations of genomic tools for guiding conservation and crop improvement strategies in a changing climate.



Plasticity, local adaptation and maladaptation to climate in a wild relative of the cultivated apples

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MEETING ROOM 114, August 19, 2025, 11:00 AM - 1:00 PM

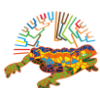
Understanding how perennial species respond and adapt to climatic variation is essential to predicting plant resilience in changing environments. While plastic responses allow individuals to cope with environmental heterogeneity, they may also mask or facilitate local adaptation, especially in long-lived species with broad distributions.

In this study, we combined field experiments, transcriptomics, and population genomics to assess the relative roles of phenotypic plasticity, gene expression divergence, and adaptive potential in the domesticated apple (*Malus domestica*) and its wild relatives *M. sylvestris* and *M. orientalis*. We grew 894 seedlings from 46 populations in four common gardens simulating distinct climates and measured 12 functional traits related to growth, resource allocation, and stress. Gene expression was quantified in 96 seedlings under contrasting conditions, and population-level variation in gene expression plasticity was linked to SNP-based genome–environment associations and estimates of mutational load.

Climate strongly shaped seedling growth and physiology, explaining most of the phenotypic variance across species. All species displayed high plasticity, but *M. orientalis* showed greater phenotypic stability across environments than *M.*

sylvestris, while domesticated apple was highly plastic but suffered reduced performance in stressful environments. Genes differentially expressed between climates (climate-DEGs) were highly modular, enriched in nutrient and defense functions, and partially overlapped with loci showing signatures of climatic adaptation from GEA analyses, suggesting a genomic basis for adaptive plasticity. Finally, analysis of putatively deleterious mutations revealed a lower mutational burden in *M. domestica* compared to wild relatives across all gene categories. Notably, climate-DEGs carried fewer deleterious variants than non-DEGs, implying stronger purifying selection on environmentally responsive genes. The Romanian *M. sylvestris* population consistently harbored the highest genetic load, possibly reflecting demographic bottlenecks and reduced efficacy of selection.

Together, our findings show that climate-driven plasticity in apples is widespread but structured, involving genes under stronger selection and contributing to differential resilience across populations. This integrative framework combining ecology, expression, and genomics helps clarify the complex interplay between plasticity, adaptation, and genetic load in perennial fruit trees.



Spatial scales of genomic adaptation to climate and phenology in the winter moth

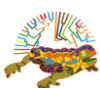
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MEETING ROOM 114, August 18, 2025, 10:30 AM - 12:30 PM

Climate change is altering global ecological conditions, and a key challenge is understanding how quickly populations can adapt to rising temperatures. Seasonal timing traits provide some of the best evidence of phenotypic responses to climate change, including rare but compelling examples of genetic adaptation. When trophic interactions impose strong selection on phenological traits—as in the case of winter moth larvae needing to hatch in synchrony with deciduous tree leaf-out—they offer powerful models for studying evolutionary responses. However, we still lack a clear understanding of the spatial scale, genomic architecture, and environmental drivers of such adaptation. Here, we investigate the genomics of adaptation to climate and host phenology in

the winter moth (*Operophtera brumata*), an important prey species for insectivorous birds like the great tit. We sequenced 151 whole genomes from 16 sites across the UK, encompassing a wide range of spatial scales (pairwise distances from 1 to 1100 km), altitudes (from sea level to 200 m), and climatic conditions (mean spring temperature range: ~7–17°C). We first assess population structure and model dispersal to determine the scale at which local adaptation might occur. We then combine gene–environment associations, selection scans, and structural variant discovery to uncover the genomic basis of adaptation. Our results provide new insights into the evolutionary potential of seasonal timing traits and the capacity of species to respond to rapid environmental change.



Speciation under stress: Genomic and ecological divergence in Eastern Baltic Cod

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MEETING ROOM 114, August 18, 2025, 10:30 AM - 12:30 PM

Marginal populations at the distribution edge of a species are often more vulnerable to environmental stressors than core populations. When such marginal populations are exposed to additional human-induced impacts—such as overfishing, habitat degradation, and climate change—they may suffer disproportionate declines, particularly if they are already ecologically and genetically distinct from neighboring groups. The Eastern Baltic Cod (EBC) exemplifies above scenario and has experienced a severe and ongoing collapse marked by the loss of spawning grounds, sharp declines in population size, body condition, and body size, likely driven by intense fishing pressure and challenging environmental conditions such as increasing hypoxia. Having diverged from the Western Baltic cod approximately 7,000 years ago, EBC has since rapidly adapted to the unique environmental conditions of the Baltic Sea such as low salinity and oxygen level. Evidence suggests strong reproductive

barriers between the two populations, including mismatches in spawning time and location, and the notable absence of adult hybrids in the wild—hallmarks of ongoing speciation. In this study, we synthesize existing knowledge on reproductive isolation and apply genomic tools to identify and quantify hybridization across life stages. Leveraging a new reference genome for EBC, we also investigate genome-wide divergence and infer the population's demographic history. Recognizing EBC as a genetically distinct species has critical implications for management and conservation. If reproductive isolation is strong, this population cannot be replenished from the west upon collapse. Thus, reframing EBC as a separate evolutionary unit calls for urgent reassessment of current stock management practices and the development of conservation strategies that reflect true population boundaries.



Strong Selection, but low repeatability: Temperature-specific effects on genomic predictions of adaptation

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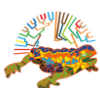
MEETING ROOM 114, August 19, 2025, 11:00 AM - 1:00 PM

Background: Climate change is causing major shifts in species' distributions, interactions, and growth rates across the globe. Predicting these shifts is thus of fundamental ecological and economical importance but remains a major challenge. One particularly promising avenue for making forecasts is to use genomic methods, such as "genomic offsets", to predict the level of (mal)adaptation and evolutionary potential of wild populations which' demography and life-history is hard to monitor, but for which whole-genome DNA sequences can be attained. However, the accuracy of such methods is currently debated and seldom validated.

Rationale: Climate warming is currently increasing temperatures beyond the optima of many ectotherms, placing many natural populations under risk and emphasising the need to evaluate our predictive power to forecast species responses to future warming climates. However, the strong selection on basal physiological and metabolic processes imposed by hot temperature also suggests that, when it happens, adaptation to warming should be relatively fast and predictable. Here, we evaluated the potential to predict adaptation

to hot and cold temperature, from the level of single nucleotide polymorphisms to life-history traits, by conducting an evolve-and-resequence experiment using replicate strains of three geographically separated populations of the cosmopolitan insect pest, *Callosobruchus maculatus*.

Findings: Indeed, adaptive evolution of life-history traits was faster and more repeatable at hot temperature. However, while genomic adaptation to heat was repeatable and showed signs of gene re-use across replicate lines of the same genetic background, it was much less repeatable than cold adaptation when compared across genetic backgrounds. As a result, genomic predictions of phenotypic (mal)adaptation to hot temperature were accurate when applied to populations of the same geographic origin but were highly inaccurate when applied to populations of different geographic origin. These results seem best explained by an increased importance of genetic redundancy and epistasis during adaptation to heat and imply that genomic predictions of adaptation in key life-history traits, which mediate the agricultural impact of insect pests, may become increasingly difficult as climate warms.



The genetics of thermal adaption in tropical butterflies

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MEETING ROOM 114, August 18, 2025, 10:30 AM - 12:30 PM

Background: Climatic stratifications, in particular differences in temperature, along altitudinal clines, can lead to local adaptation and genetic divergence between populations at different altitudes. Year-round stable temperatures in the tropics make altitudinal temperature variation more pronounced and increase the likelihood of local adaptation across relatively narrow gradients. This, combined with tropical species often living close to their thermal maximum, has been used to argue that tropical species may be particularly threatened by increasing temperatures. However, genetic variation present within species across altitudinal clines may also provide the necessary raw material for natural selection to act on in response to changing climates.

Methods: Here we focus on two butterfly species, *Heliconius erato* and *Heliconius melpomene* which co-occur from sea-level up to just over 1000 meters above sea level on the slope of the Andes. We used controlled rearing identify and tease apart

phenotypic variation due to genetic differences between populations and due to the plastic effects of rearing temperature as well as traits that show genotype-by-environment interactions. We then conducted transcriptomics to understand the genes underlying these differences.

Results: We found evidence of developmental, morphological, and thermal tolerance variation associated with location of origin, rearing temperature, or an interaction between these. In particular, *H. erato* showed strong evidence of adaptive plasticity in response to heat, with increased heat knockout times at higher rearing temperatures, but only in the populations from low altitude. Our transcriptomic results have identified heat shock proteins that may be responsible for this variation.

Conclusion: These results provide new insights into thermal adaptation in tropical insects and the genetic basis of plasticity, which may prove adaptive as species respond to changing climates.



Unveiling future maladaptation and preadaptation in locally adapted Mediterranean beech populations under climate change

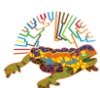
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MEETING ROOM 114, August 18, 2025, 2:00 PM - 5:00 PM

European beech (*Fagus sylvatica*) exhibits a broad distribution across Europe and has undergone local adaptation in many populations, enabling its persistence across diverse climatic conditions. We analysed eighteen Catalan populations using landscape genomics. We uncovered weak but structured genetic differentiation, revealing three main genetic provenances. Leveraging Genotype-Environment Association (GEA) methods, we also examined adaptive potential, identifying linear (LFMM, BayPass, RDA) and non-linear (Gradient Forests) relationships between allele frequencies and environmental variables. We identified numerous Single Nucleotide Polymorphisms (SNPs) correlated with environmental gradients, with an over-representation of gene classes associated with drought and heat stress responses. This suggests that genes regulating key physiological processes are involved in climate adaptation. By applying Gradient Forest, we further mapped allelic turnover across the region and delineated three distinct genetic zones. Furthermore, we projected future genetic offset (2061–2080) under all the climate scenarios available for this period, pinpointing regions at heightened risk of maladaptation and

reduced fitness. Due to the greater climate shift predicted in the Pyrenees, offset values were also higher there than in lower latitude forests, and specifically we observed the highest offsets in transition areas between valleys and ridges. We also calculated the slope of the genetic offset (Slope = Genetic offset / Environmental offset), obtaining a normalized offset that let us compare populations identifying the ones that are more preadapted to their future climate. This index also allowed us to recognize which populations are the more idoneous for a translocation experiment in a Pyrenean population, being those the ones more preadapted and with a lower offset to the future climate of the receiving population. Our results highlight the drought-temperature gradient as the primary driver of allele frequency shifts, alongside an isolation-by-distance pattern shaped by geographic distribution. These results underscore the importance of considering standing genetic variation in conservation and forest management strategies to mitigate the adverse effects of climate change, which threatens global biodiversity and may lead to local extinctions near the species' drought tolerance limit.



Using Landscape Genomics to Predict Climate Change Vulnerability in a Large Mammal Grazer

Tengstedt A¹, Tanderup S¹, Hansen M¹

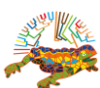
¹Department of Biology, Aarhus University

MEETING ROOM 114, August 19, 2025, 11:00 AM - 1:00 PM

Understanding how species respond to rapid environmental change is essential for effective conservation. Yet, for highly mobile, wide-ranging species inhabiting heterogeneous landscapes, identifying populations most vulnerable to climate change remains a major challenge. Here, I present the methodological framework and preliminary results from an ongoing landscape genomics study of caribou (*Rangifer tarandus*), a keystone Arctic herbivore exhibiting pronounced ecological diversity and experiencing population declines in many regions. The project integrates whole-genome sequencing data from approximately 350 individuals across North America, Greenland, and Eurasia with spatially explicit environmental variables to evaluate local adaptation and predict future genomic vulnerability.

Key components include: (i) genotype–environment association analyses to identify loci involved in local adaptation; (ii) genomic offset analysis to predict populations at risk of maladaptation; and (iii) genomics-informed species distribution models (SDMs), which combine environmental predictors with adaptive genomic data to improve projections of range shifts and population persistence under climate change.

By aligning genomic variation with ecological gradients and projecting potential adaptive mismatches under future scenarios, this approach aims to bridge the gap between evolutionary insight and practical conservation management. I highlight how genomic insights may guide assisted gene flow and adaptive management in caribou populations.





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S24 - Forecasting evolution in natural populations



Adaptive Evolution in Brassicaceae: Unraveling Mechanisms of Genomic Convergence in Serpentine Adaptation Across a Broad Range of Genetic Divergence

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POSTER SESSION 3 - **P03.056**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Convergent or repeated evolution—the independent evolution of similar phenotypes, ecological strategies, and molecular pathways—provides an opportunity to investigate the predictability of evolution. Previous research has shown that gene reuse in adaptation decreases as divergence between lineages increases, yet the extent of genomic convergence and how it scales with genetic divergence remains unclear at deeper evolutionary timescales. We address this gap by exploring the genetic basis of repeated adaptation to highly selective serpentine soils across ~25 million years of divergence in the plant model family Brassicaceae.

Using genome-wide selection scans, we aim to identify candidate adaptive genes in 10 Brassicaceae species by comparing serpentine-adapted populations with their non-serpentine counterparts. Serpentine soils represent a strong selective pressure due to an extremely skewed Ca/Mg ratio, high heavy metal concentrations, and a shortage of key nutrients. By compiling species-specific lists of likely adaptive genes for this environment and analyzing their overlap, we will evaluate the

extent of gene reuse and determine how it scales with evolutionary distance.

We expect that the decline in gene reuse at shallow taxonomic levels is primarily driven by limited allele sharing, where two or more populations retain the same adaptive allele through shared standing genetic variation or gene flow. However, the role of de novo mutations in shaping gene reuse remains less explored. Through a systematic analysis across species and genera, we aim to unravel the contributions of allele sharing versus de novo mutations, clarifying the mechanisms underlying repeated adaptation across increasing divergence scales.

Here we present our first results on population structure and the extent of serpentine candidate gene sharing across genera, *Cardamine*, *Odontarrhena* and *Alyssum*, suggesting a decline in gene reuse with increasing divergence time. Altogether, our study aims to contribute to a broader understanding of genomic convergence and repeatability and the factors shaping adaptive evolution in natural populations.



Evolution of a marine invertebrate in urban coastal habitats

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POSTER SESSION 3 - P03.063, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Marine species experience severe modifications of their habitats in harbours and marinas, which differ from neighbouring natural habitats by multiple biotic and abiotic factors. Urban coastal zones therefore provide interesting situations to study the adaptation of marine species to anthropogenic habitat modifications. Here, we studied the marine gastropod *Littorina fabalis*, a species that is established in many marinas and that, in its natural habitat, presents two ecotypes established along meter-scale wave exposure gradients. We use this system to test whether responses to the anthropogenic gradient are similar to those found in nature and to look for genomic signatures of selection private to the urban environment. More precisely, we hypothesized that pre-existing adaptive genetic variation in wild population, notably at chromosomal inversions, has allowed rapid adaptation to the environmental contrasts created by human hands from inside to outside a harbour.

Methods: We sampled two transects covering populations established along a wave exposure gradient both in a natural and a harbour site located 30km apart in Western France. Around 150 snails were collected

along each transect. Whole Genome Sequencing (WGS) data was then analysed to study the signature of selection along the transects, and explore the role of chromosomal inversions for adaptation along a recently established artificial habitat shift (that was built 75 years ago).

Results: We found that the genomic landscape of differentiation between sheltered and exposed areas was nearly identical when comparing natural conditions to artificial settings (from the inside to the outside of a marina). Most genetic differentiation clustered within the 15 already described chromosomal inversion polymorphism. However, the change in frequency of the inversion's arrangement were much more abrupt in the harbour site, with much of the genetic variants differentially fixed over just a few meters at the entrance of the harbour (a pattern never observed so far in natural populations).

Conclusion: These results suggest that inversions responses to selection are stronger in harbour. The implication of these results to our understanding of the evolutionary responses to rapid anthropogenic changes will be discussed.



Fluctuation of population abundance over decade in one amphibian assemblage

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POSTER SESSION 3 - **P03.057**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Amphibians are the most endangered vertebrates. Evidence on their habitat deterioration and loss suggests future population declines and critical challenges in maintaining species' genetic diversity. Long-term monitoring of local amphibian populations provides essential data for understanding their microevolutionary dynamics, including impact of genetic drift and adaptive responses to environmental stressors. Neglecting monitoring of common species might result in many silent local extinctions and in loss of information how population bottlenecks impact on evolutionary potential. Population size can be negatively influenced by climate change, particularly affecting early spring breeders. We used data on population abundance of three syntopic anuran species, collected over the years, to recognize possible loss of genetic diversity.

Materials and methods: Starting from 2011, we monitored three anuran species (*Bufo bufo*, *Rana dalmatina* and *Pelophylax esculentus* complex – PEC) in a local pond. Annual fluctuations in individual counts (N) were recorded for *B. bufo* and PEC, while for *R. dalmatina* we used egg clutches counts instead. For early breeders (*B. bufo*, *R. dalmatina*) reproductive timing was recorded. The COVID-19 interruption in 2020 necessitated predictive approximation based on the mean values from other years for reproductive season endpoints. Kendall Tau correlations were implemented to check if the parameters have trends across the years. Effective population size (N_e) and N_e/N ratios were calculated for *B. bufo*, as quick estimation of possible genetic variability loss. We applied LassoCV regression with 5-way cross-validation (23 variables) to describe what impacts on the length of reproductive season of this *B. bufo* local population.

Results: All three species exhibited annual fluctuations of population abundance, PEC

being the least variable in numbers; Kendall Tau showed no trends over years. *B. bufo* showed a constantly low female count (4–44 males per female), N_e values from 23 to 548 and N_e/N ratios from 0.09 to 0.71. Optimum alpha value for LassoCV was 0.069921482. Time needed for the number of males to drop from maximum to 0 had the greatest positive impact on duration of reproductive season (coefficient = 7.32). Time males needed to gather from the few to the maximum numbers had high positive impact (coefficient = 2.42). Time females spent in the reproductive pond had moderate positive impact (coefficient = 1.60). Time males spent there after females' departure had low positive impact (coefficient = 1.01). Difference between the date of maximum female and maximum male numbers had very low positive impact (coefficient = 0.04). Number of males had very low negative impact (coefficient = -0.001).

Conclusions: In local populations of three anuran species only fluctuations in numbers were detected. The male-biased *B. bufo* population risks allelic loss (accelerating genetic drift and inbreeding) and reduction of adaptive potential. N_e and N_e/N were typical for amphibians, with N_e/N being critically low in 2017 (0.09), indicating bottleneck. Males drive the reproductive season in *B. bufo* local population as they come first and stay after females, sometimes for a long period. Males would reach maximum numbers up to eight days later than females. Unexpectedly, start day of reproductive season didn't have any impact on the duration of reproductive season. The stable PEC population abundance suggests maintenance of genetic diversity, what buffers against environmental stochasticity. Species with stronger population fluctuations face possibility of genetic diversity loss.



How the common evening brown butterfly conquered the world

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POSTER SESSION 3 - P03.065, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Species capable of colonizing a wide range of habitats can become widespread and then encounter various environments, to which populations may evolve local adaptations. The common evening brown butterfly, *Melantis leda* (Lepidoptera: Nymphalidae), is found in almost all tropical and subtropical regions of the Old World, using forests and wooded savannah. We here aim to 1) reconstruct its global phylogeography, 2) determine population structure, and 3) infer the demographic history of populations in various regions.

Materials and methods: To cover the wide distributional range of *M. leda*, we collected DNA samples from 21 countries across three realms: Afrotropics, Indomalaya, and Australasia. We first created a reference genome and then used Illumina short-read

whole genome sequencing for 5 – 10 samples for each location at 20x coverage. We then mapped these sequences to the reference genome to identify single-nucleotide polymorphisms (SNPs). We used SNPs from the nuclear genomes to reconstruct the global phylogeography and gene flow among populations. We then estimated historical variation through time in effective population size for each population.

Results: Our results suggest that the common evening brown originated in mainland Asia, from which it colonized Australasia and, more recently, Africa. These results will provide a basis for elucidating the role of climatic variation in range expansion and population size, as well as tracing the footprints of adaptation to climate and habitat in the genome.



Local Adaptation in Baltic Cod? Insights from Early Development

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POSTER SESSION 3 - P03.058, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

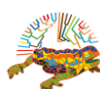
Atlantic cod (*Gadus morhua*) lay pelagic eggs that must be buoyant to develop. Baltic cod, phenotypically and genetically distinct from Atlantic cod, are uniquely adapted to the brackish Baltic Sea. Eggs from the eastern Baltic stock are buoyant at >14-15 psu, salinities found only in the south and central Baltic Proper, their historical spawning grounds.

However, laboratory experiments have shown that some Baltic cod eggs can develop at salinities as low as 7 psu, with a high survival rate at 9 psu. This environment exists in the northern Baltic Proper, in the Åland Sea, where cod are large, healthy, and in breeding condition. Yet, eggs from both northern and southern populations are negatively buoyant at these salinities, raising the questions: i) are northern cod better adapted to low salinity and ii) have they developed demersal spawning?

This study examined whether the northern population is better adapted to northern conditions than the southern population. We assessed larval survival and neutral

buoyancy after incubation at 17 psu (control), 9 psu, and 7 psu, expecting northern cod to outperform southern cod showing higher survival at low salinity and more buoyant larvae. Additionally, we tested whether Baltic cod eggs hatch from sediment.

Results show a decrease in survival rate at the lowest salinity treatment, but equally between the two populations. Neutral buoyancy differs between the salinity treatments but not between the populations, suggesting that larvae from both populations show similar physiological responses to the salinity decrease. Interestingly, northern eggs hatched from sediment, but so did southern eggs, without differences from the control (non-sediment) treatment. These results do not support the hypothesis of local adaptation to the northern Baltic environment and thereby a divergence between the two populations, but do not discard the possibility of cod being able to reproduce outside what is considered the historical spawning ground.



omniCADD: prediction variant deleteriousness beyond model organisms

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POSTER SESSION 3 - **P03.060**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Many animal populations across the globe are currently experiencing significant declines due to environmental, ecological, and human-induced pressures. As populations decrease in size, their genetic health becomes increasingly vulnerable. Smaller population sizes are particularly susceptible to inbreeding, which can elevate the prevalence of deleterious alleles. This loss of genetic diversity reduces a population's ability to adapt to environmental changes, resist diseases, and endure long-term, ultimately increasing the risk of extinction. The genetic implications play a vital role in conservation biology and highlight the necessity to enhance our understanding and monitoring of the genomic health of endangered species. Genomic studies often focus on species with extensive genomic resources, known as model organisms, but many declining species are non-model organisms for which such resources are scarce. Therefore, developing reliable methods for assessing genetic health across a wide variety of species is essential.

Materials and Methods: To address the need for broader genomic monitoring tools, we sought to develop a predictive model capable of identifying potentially harmful genetic variants in both model and non-model organisms. Building upon earlier frameworks (Combined Annotation Dependent Depletion; CADD) that assessed variant deleteriousness within a single species, we aimed to create a more flexible and widely applicable approach. Our initial work focused on domesticated pigs (*Sus scrofa*), for which extensive genomic data are available. Using deep sequencing data from a diverse cohort of pigs, we trained a machine learning model to distinguish between benign and deleterious mutations based on a variety of genomic features. These features included conservation scores, variant effect predictions, and amino acid substitution scores.

The next phase of our study involves testing the model's transferability by applying the same methodology to wild boars (*Sus scrofa*),

functioning as the wild equivalent of domesticated pigs. This comparison will help us evaluate how well a model trained on domesticated animals performs when applied to genetically related but ecologically distinct populations. If successful, the methodology could be extended to truly non-model organisms, increasing its utility in global conservation efforts.

Results: Our preliminary model trained on domesticated pig genomes demonstrated equal accuracy in predicting deleterious variants when evaluated against the previously developed pig-specific model pCADD. The model was able to effectively differentiate between likely harmful and neutral mutations, offering a valuable tool for assessing the genetic health of pig populations. We are currently in the process of retraining the model using genomic data from wild boars, applying the same feature set and machine learning algorithms. The model can be run on species with high(er) resolution data, with the adaptation to new genomes ongoing. By directly comparing the performance of both models, we aim to assess the impact of ecological and evolutionary differences on model generalizability.

Conclusions: The development of predictive models for identifying deleterious genetic variation represents a promising direction in conservation genomics. By extending this approach beyond well-studied model species, we can improve our ability to monitor and manage the genetic health of endangered or vulnerable populations. This, in turn, may allow for more informed conservation strategies, including genetic rescue or habitat management interventions. Ultimately, our work contributes to a growing effort to integrate genomic data into conservation decision-making, to prevent further biodiversity loss, and to better understand the genetic underpinnings of species decline and extinction.



The link between Doubly Uniparental Inheritance (DUI) of mtDNA and sex determination in marine mussels: a unique case of sexual system evolution from dioecy to trioecy

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POSTER SESSION 3 - **P03.066**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Mitochondrial DNA (mtDNA) inheritance in most eukaryotes follows strict maternal transmission, where the mitochondrial genome is passed exclusively from mothers to offspring. However, certain taxonomic groups, such as marine and freshwater mussels, exhibit an atypical mechanism known as doubly uniparental inheritance (DUI). In this system, males inherit mtDNA from both parents, while females inherit only maternal mtDNA, resulting in the coexistence of two distinct mitochondrial lineages (M and F-type) within a species. The M-type mitogenome, paternally transmitted and predominantly found in males, and the F-type mitogenome, maternally transmitted and dominant in females, lead to heteroplasmy in males and homoplasmy in females. In DUI species, M- and F-type mitogenomes show significant genetic divergence (up to 40%).

The evolutionary role of DUI remains debated, but recent hypotheses suggest its involvement in sex determination. Studying mussel species with diverse reproductive strategies provides a unique opportunity to explore the potential link between DUI and sexual differentiation. Here, we investigate mtDNA inheritance and sexual system evolution in two closely related marine mussels with contrasting reproductive strategies: the trioecious *Semimytilus patagonicus* (coexisting females, males, and simultaneous hermaphrodites) and the dioecious *Perumytilus purpuratus* (separate sexes in a 1:1 ratio, with rare intersex individuals).

Materials and Methods: Mitochondrial DNA was sequenced from gonadal tissue of wild-caught hermaphroditic and unisexual *S. patagonicus*, and unisexual and intersex *P. purpuratus* specimens using the E.Z.N.A.® Tissue DNA Kit. Libraries were prepared (TruSeq Nano) and sequenced (Illumina NovaSeq, 151 bp paired-end), yielding 30 Gb of

data. After quality trimming (Q20, removal of reads <40 bp), reads were mapped to M/F-type reference mitogenomes (CLC Genomics Workbench v.24.0.1) under stringent parameters. Species-specific primers were designed for validation.

Results. We confirmed DUI in both species. In *S. patagonicus*, hermaphrodites exhibited heteroplasmy in male gonadal tissues (M+F mitogenomes), while female tissues were homoplasmic (F-type only). Unisexual individuals (males/females) lacked DUI: males carried only M-type, and females only F-type. Comparative mitogenomic analysis revealed structural differences: male M-type genomes had repetitive sequences (particularly near rRNA genes) and deletions in ND2, ND6, ND4, CYTB, and COX3.

In *P. purpuratus*, dioecious individuals followed classic DUI (heteroplasmic males, homoplasmic females), but intersex individuals lost DUI, with male gonadal tissues harboring only M-type and female tissues only F-type. Intersex individuals also showed mitogenome synteny differences.

Conclusions. Our results demonstrate an association between doubly uniparental inheritance (DUI) and sexual differentiation, particularly in hermaphroditic and intersex individuals. The structural reorganization of rRNA genes and accumulation of repetitive elements in M-type mitogenomes suggest their potential functional involvement in mitochondrial gene regulation during sex determination. DUI appears to facilitate sexual plasticity in these organisms. This study provides critical insights into mitochondrial inheritance's role in bivalve sexual differentiation and advances understanding of evolutionary mechanisms behind sexual plasticity in organisms with atypical mtDNA inheritance—a unique case in Animalia. Fondecyt 1251077



The role of developmental plasticity in the resilience of spionid populations

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POSTER SESSION 3 - **P03.064**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Alongside mutation, plasticity is a key driver of phenotypic diversity within populations. It creates diversity by producing different phenotypes from a single genotype, depending on the environmental cues an individual receives. Producing phenotypes through adaptive plasticity means that individuals may be able to match their phenotype as close as possible to the environmental optimum, maximising fitness. Such phenotype matching often explains why some species are particularly effective at invading novel environments, whilst others are not. At the population level, plasticity can mitigate selection by buffering individuals against selection. This has significant consequences on evolution, since plastic populations will possess more phenotype variance which can be selected on, in addition to having more cryptic genetic variation than non-plastic populations. However, aside from being used to explain the prevalence of plasticity among invasive species, the buffering effects of plasticity on populations have rarely been tested outside of a lab-setting. Such a research gap brings

into question whether we can observe any tangible differences between plastic and non-plastic species in their population persistence or extirpation. To examine this, we use a timeseries of spionid species abundance across the Wadden Sea, since it is both a long-term (15 year) and high spatial-resolution dataset. Using this system, we ask whether populations of plastic species are more resilient to environmental change than non-plastic species, before going on to examine whether plastic species perform better in highly varied environments than non-plastic species. We find that plastic species experience less variance in population size than non-plastic species, indicating significant buffering and suggesting that these plastic species may have a greater capacity to adapt plastically and evolutionarily to changes in their environment. We conclude by discussing the implications of this for evolution amongst spionid populations, and potential uses for this system in examining eco-phenotypic feedbacks.



Where have all the flowers gone? What could we start learning about possible evolutionary patterns from multi-year study of population dynamic of sexual and vegetative reproduction in clonal *Iris pumila* natural population in Deliblato Sand

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POSTER SESSION 3 - **P03.062**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

To forecast changes in natural populations we must consider multiple factors including environmental ones, and identify components of our predictive framework that are most in need of improvement. These issues can be approached through the empirical analysis of natural populations of existing model systems.

Such a system that has been studied for the last 35 years is the dwarf bearded iris, *Iris pumila* L. (Iridaceae), a small monocot that occurs in the lowlands of central and eastern Europe. Being a perennial clonal plant that spreads vegetatively through underground rhizomes („phalanx“ strategy of spread), *I. pumila* also possesses hermaphroditic, entomophilous flowers, and is a strictly outcrossing species. One of the most astonishing features of *I. pumila* is its huge flower color polymorphism that exist in each population, and studies explaining this kind of phenomenon are scarce. Flower color polymorphism and species population dynamic are closely connected and, in perennial plants with both vegetative and sexual reproduction, all those components should be jointly considered in selection analyses, as well as their spatial and temporal variability patterns.

In *I. pumila* natural population situated in Deliblato Sand Natural Reserve (40 km northeast of Belgrade, Serbia) we placed twenty-five 12 x 12 m grids and ten 7 x 7 m grids, leading to total of 4090 square meters covered. During 2013-15 flowering seasons we recorded occurrence of all flowers in each of 4090 selected square meters by means of digital color photography. Since 2021, data collection was considerably enhanced by utilization of DJI Mavic Pro drone with Hasselblad L1D-20c aerial camera. This left enough time for simultaneous sample collection for reflectance UV enhanced spectroscopy (since 2021), as well as for HPLC and microsatellite analyses (since 2024), preliminary results of which would be separately presented at ESEB 2025.

Preliminary analysis of ten 7 x 7 m grids presented in this work revealed huge differences between flowering seasons in flowering phenology. While differences between flowering onsets and peaks in different flowering seasons can be traced down to different environmental conditions, reasons and mechanisms for observed constant decline in number of flowers (from several thousands to the only couple of hundred flowers in the subsample) is a component of our predictive framework that is in the need of further investigation. To begin with, preliminary analyses clearly indicated switches between reproductive modes since decline was mostly due to lesser percentage of individual ramets that produced flowers, compared to much smaller decline as a result of reduction in number of *I. pumila* individual plants.

Those analyses should be expanded to the whole sample and further combined with results of future flower color and genetic analyses, since interplay between sexual and vegetative reproduction in self-incompatible species crucially depends on spatial genetic composition. Those lines of research can then have impact on our understanding of possible evolutionary changes in natural populations of *I. pumila*.

Acknowledgements

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Divergence time and environmental similarity shape the repeatability of morphological evolution in stick and leaf insects.

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MEETING ROOM 129+130, August 22, 2025, 10:30 AM - 12:30 PM

The observation of repeatability in phenotypic evolution across taxa suggests that, at least sometimes, evolution may be forecasted. Yet it is still unclear what factors determine the level of repeatability (and, by extension, predictability) of evolutionary outcomes and trajectories. If independent lineages are subjected to the same environmental challenges, what factors influence how often they evolve the same phenotypes? When does repeatability break down?

One possibility is the genetic distance between lineages: closely related lineages will share more standing genetic variation, genetic background and developmental mechanisms and are therefore expected to respond in a more analogous way to similar selective regimes. Another possibility is the actual degree of environmental similarity and therefore the extent to which the selective regimes are similar between independent lineages occupying qualitatively and seemingly identical environmental niches.

We tested the effects of these two factors using stick and leaf insects (order Phasmatodea) lineages that repeatedly transitioned to similar habitats. Phasmids are renowned for their extraordinary morphological diversity associated with camouflage, ranging from elongated branch

mimics to wide and flattened leaf mimics. We used morphological measurements for 212 species spanning all major clades to reconstruct a multidimensional morphospace which, in combination with a robust phylogenetic reconstruction, was used to show the repeated evolution of many ecomorphs in the group. We show that when transitioning to the same habitat, stick insect lineages that are more closely related and experiencing more similar environmental conditions end up displaying more similar morphologies at present. More closely related lineages also display more parallel trajectories on the morphospace following the transition.

Our work sheds light on two critical factors that influence the level of predictability of phenotypic evolution. Our findings directly evidence both the contingent and deterministic nature of evolution: independent lineages evolve in more analogous ways when submitted to more similar environmental pressures (determinism) and when they are less genetically divergent (contingency). We show that the extent of repeatability decreases at a relatively constant rate across more than 100 My of divergence, suggesting that even the magnitude of contingency can be predictable, given sufficient spans of time.



Jointly forecasting adaptive evolution and demography: refining tools and semantics.

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MEETING ROOM 129+130, August 22, 2025, 10:30 AM - 12:30 PM

Forecasting evolution in natural populations is an important task, and one that often benefits from a complementary demographic perspective. Indeed, accurately predicting evolution in stage-structured populations requires accounting for demographic structure. Further, understanding the demographic consequences of evolution is a major goal in the context of unprecedented environmental changes. Finally, demography can also feedback on evolutionary processes, which may affect forecasts.

To help with these goals we developed a new Evo-Demo Hyperstate Matrix Population Model (EvoDemo-Hyper MPM) that incorporates the two-sex genetic inheritance of quantitative traits, enabling fast computation of projected evolutionary and demographic dynamics under dynamic environments. EvoDemo-Hyper MPM overcomes some limitations of previous matrix Evo-Demo models, in particular in relation to the representation of genetic

inheritance. EvoDemo-Hyper MPM matches outcomes from individual-based simulations and analytical approximations under simple biological conditions, but is much faster than individual-based simulations and more flexible than analytical approximations. I will illustrate the method with empirical evolution-demography forecasts for a wild population of wandering albatrosses experiencing effects from climate change.

While developing the model we identified major semantic differences between fields, which may explain misunderstandings between researchers from different fields working on the same question. I will present the different points of view and show that they produce equivalent results, but express them on different scales. Ultimately, progress on forecasting evolution requires integrative approaches, and with it for researchers from different traditions to understand each other.



Quantitative prediction of population mean fitness evolution: intra- and inter-sexual genetic (co)variances measure the expected amount of adaptive change to survival and reproduction in the face of sexual conflict and competition

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MEETING ROOM 129+130, August 22, 2025, 10:30 AM - 12:30 PM

Predicting how fitness evolves in a population is a central objective in evolutionary biology and population ecology. Total change in fitness can be formalized using the Price Equation to partition population mean fitness change due to natural selection from other evolutionary and environmental causes. However, most studies to date have invoked Fisher's Fundamental Theorem of Natural Selection to predict the rate of population mean fitness change due to natural selection as a function of the additive genetic variance in fitness, thereby leaving out abiotic or biotic changes in the environment, such as competition, and implicitly assuming hard selection. Despite a long history of theoretical development, there has been comparatively little empirical testing of either the Price Equation or Fisher's Fundamental Theorem to make quantitative predictions of future changes in population mean fitness. We used experimentally manipulated, replicate populations of seed beetles (*Callosobruchus maculatus*) to empirically parameterize the Price Equation and Fisher's Fundamental Theorem of Natural Selection and evaluate our ability to use them to make prospective predictions for the rate of change in population mean fitness.

In each replicate we applied a complex breeding design over multiple generations

coupled with individual survival and competitive mating success assays to enable fitting a quantitative genetic model that accounts for sex-specific direct and indirect (i.e., competitive effects) additive genetic variances and covariances for lifetime survival and reproductive success. These analyses revealed the magnitude by which intralocus sexual conflict, estimated by the cross-sex additive genetic covariance, creates a gender load that reduces the total additive genetic variance for fitness and impedes the rate of evolution in population mean fitness. We also estimated relatively small genetic variances for conspecific competitive effects, thereby quantifying the potential for this source of environmental deterioration to reduce the adaptive potential of population mean fitness, as described by the Price Equation. By decomposing quantitative predictions for change to mean population fitness into adaptive change, realized by the systematic differences in sex-specific strategies to maximize fitness, and changes in soft selection, measured by the evolutionary potential of competitive interactions, we demonstrate the importance of quantifying key components in accurate evolutionary prediction that have heretofore been neglected.



The use of multi-response models to improve inferences about natural selection

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MEETING ROOM 129+130, August 22, 2025, 10:30 AM - 12:30 PM

Understanding the process of natural selection is a key goal in ecology and evolution, and accurate estimates of selection are essential if we are to hope to correctly predict and forecast how wild populations will evolve over time. Lande and Arnold proposed a simple linear regression-based approach to estimate selection which has allowed ecologists to measure selection on a range of phenotypic traits and revolutionized the study of evolutionary ecology. However, despite the benefits of the Lande-Arnold approach, and its widespread use for estimating selection in wild populations, issues around interpreting causality from these estimates remain. Furthermore, the potential importance of hard and soft selection, including how they can bias selection gradient estimates depending on how fitness has been standardized, remains relatively unexplored. Here we show how, when fitness and traits have been measured repeatedly across

individuals and common environments, selection gradients can be decomposed into their causal components and that this decomposition can help reveal if estimates of selection are consistent with causality and distinguish and correct for soft selection. We also show how, without additional data, multi-response models can be used achieve this decomposition and thus improve our estimates of selection in wild populations. We demonstrate these methods using an empirical example in a wild population of red deer and highlight the difference this makes to our estimates of selection, and our understanding of them, compared to those estimated using the Lande-Arnold approach. Given the relative ease with which multi-response models can be applied, we hope that our illustration of this approach will allow application across a range of systems and thus improvement of our estimates and ultimately our understanding of selection and evolutionary processes.





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S25 - Gene Content Across Genomes: Models and Genomic Data



A high-quality genome for *Pagodroma nivea* facilitates understanding the evolution of highly adapted 'tube-nosed' (Procellariiformes) seabirds

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POSTER SESSION 3 - **P03.073**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

To enable more informative comparative genomics, more high-quality genomic resources are needed. We present the most contiguous genome generated for a petrel, albatross, or shearwater (order Procellariiformes), generated through PacBio HiFi sequencing complemented with short-read and Hi-C analyses. We sequenced one of the southernmost breeding species of birds from this order, the snow petrel (*Pagodroma nivea*). Snow petrels are endemic to the Antarctic mainland and surrounding sub-Antarctic islands and widely distributed in that habitat. They are one of only three species that have been seen at the south pole. Like all Procellariiformes, they have a tubular nasal passage that enhances olfaction, and they drink seawater, necessitating salt regulation, aided by their nasal anatomy. Species in this group forage over a broad oceanic range, facilitated by their enhanced olfaction which likely aids both finding prey and navigating back to their home colonies. We are interested in the genomic adaptations associated with these enhanced faculties. Using our newly generated genome and after annotating available Procellariiformes and

other seabird genomes, we compared gene family expansion and contractions. For example, Procellariiformes and penguins have 30 significantly contracted genes compared to other seabirds. Furthermore, select genes involved in esterification, osmolytes, ion transport, and the family of olfactory receptor loci were investigated. We also compared our new snow petrel genome to genome assemblies for other bird species from the Austrodyptornithes clade. With respect to patterns of genome structure, while the longest four chromosomes were highly conserved, rearrangements were more common on the smaller chromosomes. Through our long-read generated genome, we were also able to examine duplications in mtDNA that are common in birds, but difficult to detect without long read sequencing. The generation of new high-quality genomes enables comparative genomics and allows discoveries into what makes orders unique. We will discuss what our chromosome-level genome together with comparative genomic analyses tells us about the evolution of this highly adapted order of seabirds.



A phylogeny aware analysis of function for the biodiversity genomics era

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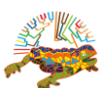
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POSTER SESSION 3 - P03.068, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

A fundamental question in biology is: when did a certain function originate? Was it recent or existent millions of years ago in the ancestors of extant species? We are currently in an era where the burgeoning amount of whole genomic data and advancements in annotation methods has given rise to an unprecedented number of complete, well-annotated proteomes from across the tree of life. It is thus possible to leverage the data available in public databases and reconstruct the detailed evolutionary history of genes, pathways and more broadly biological functions. In this study, we used the homolog and ortholog information of ~4.5 million proteins from 508 species across the tree of life and traced the emergence and evolutionary history of every gene. Next, we grouped genes by their function (GO terms/protein domain architecture) or membership to biological pathways (KEGG/Reactome) and traced the

emergence and evolutionary histories of these broader functional entities.

We found that the largest founder events of biological entities were in eukaryotes, more specifically in vertebrates, angiosperms, and slime molds. Upon inspection of GO terms and KEGG/Reactome pathways enriched at each node in the tree, we observed characteristic adaptations emerging. We also show parallel independent evolution of key genes and functions across clades with large transitions such as aquatic to terrestrial and unicellularity to multicellularity. Overall, our work is the first to elucidate the detailed evolutionary histories of biological functions using genomic annotations, which can be explored on a web application. We envision this work will be useful to a wide variety of scientists interested in the molecular history of life on earth.



Antibiotic Resistance Prediction in *Staphylococcus aureus* Using Gene Content Outperforms SNV-Based Approaches

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POSTER SESSION 3 - **P03.079**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Antibiotic resistance is a critical global health issue. Advances in genome sequencing and computational methods have enabled rapid prediction of bacterial resistance profiles. Although single nucleotide variant (SNV)-based models are often used, they may perform poorly when generalizing to novel strains due to population structure effects. Models based on whole genome sequences or specific resistance genes are also widely used, however, obtaining complete assemblies or resistance gene profiles can be challenging in rapid sequencing approaches like metagenomic shotgun sequencing from patient samples. Such methods typically generate fragmented data, providing only a broad view of the genome. In this context, clustering homologous genes and using their counts emerges as a practical alternative for resistance prediction. Here, we show that gene content can predict antibiotic resistance in *Staphylococcus aureus* and outperforms SNV-based approaches when generalizing across phylogenetic clades.

Materials and Methods: *S. aureus* genomes and antibiotic resistance phenotypes for six drugs were downloaded from the BV-BRC database, with sample sizes varying by drug (842-2681 strains). Protein-coding genes were predicted from genomes using Prodigal and clustered with CD-HIT at 80% identity. Cluster assignments were parsed into a matrix representing the number of genes from each cluster per strain. XGBoost classifiers were trained to predict resistance using gene cluster counts with an 80/20 train-test split. Clusters were iteratively removed based on XGBoost's importance by weight, retraining the model each time until the F1-score (harmonic mean between precision and recall) fell below 0.5. To investigate phylogenetic relationships among the strains, IQTree was employed to construct trees using (1) binary gene content matrices (0/1 indicating cluster absence/presence) and (2) concatenated alignments of 313 single copy core genes (present in $\geq 99\%$ of strains). Core gene

alignments were used to train additional XGBoost models, with each nucleotide encoded as an integer. To simulate generalization to novel strains, models were also trained and tested on different clades based on phylogenetic trees from core sequences.

Results: Models using gene cluster counts achieved high predictive performance, with F1-scores ranging from 0.934 to 0.995 across antibiotics. Feature importance analysis showed that resistance prediction relies on a broad set of gene clusters: contrary to our expectation that predictive scores would decay after removing few clusters, F1-scores remained above 0.5 even after removing over 30% of the most important features. On average, performance remains robust ($F1 \geq 0.8$) after removing 1232 ± 606 clusters, indicating that prediction is driven by distributed genetic signals. Phylogenetic trees based on both gene presence/absence and core gene sequences showed partial clustering of resistant and susceptible strains. Trees from core sequences also allowed identification of clades for phylogeny-aware model evaluation. SNV-based models achieved good F1-scores under random splits but showed substantial drops when evaluated on unseen clades, performing not better than random predictions. Surprisingly, gene content models maintained higher performance across the same unseen clades, with average F1-score of 0.9 across all antibiotics in all tested clades.

Conclusion: Homologous gene cluster counts can accurately predict antibiotic resistance in *S. aureus* and outperform SNV-based models in generalizing to novel phylogenetic lineages. Resistance prediction is supported by a distributed set of gene features rather than a few major determinants. Gene content-based approaches offer a robust strategy for predicting resistance, motivating future investigation of biological relationships between antibiotic resistance and a broad set of gene clusters.



Archaeorhizomycetes, a diverse group of ancient root endophytes

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POSTER SESSION 3 - **P03.088**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Our understanding of evolution is primarily informed by a few lineages, which comprise a tiny fraction of the entire tree of life. For example, it is estimated that only 1-5% of fungal diversity is currently known.

Archaeorhizomycetes (Taphrinomycotina, Ascomycota) is an understudied class of soil fungi that exemplify the difficulty of studying the diversity and evolution of most fungi, which lack macroscopic structures and are recalcitrant to culturing. Archaeorhizomycetes is a diverse and globally distributed class, with unknown ecological roles and life cycles and no observed sexual structures. Archaeorhizomycetes eDNA is frequently detected in soil and often in high abundance, regularly representing the majority of fungal amplicons. Despite extensive isolation efforts over more than a decade, only four species – *Archaeorhizomyces finlayi*, *A. borealis*, *A. notokirishimae*, and *A. ryukyuensis* – are formally described and maintained in axenic cultures. However, global richness estimates suggest the class may comprise > 15000 species (1.5% dissimilarity threshold).

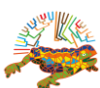
Phylogenetic analysis supports the placement of Archaeorhizomycetes as one of the distantly related lineages of the monophyletic subphylum Taphrinomycotina, with *Neolecta* weakly supported as its closest sister-lineage. *Neolecta* is a genus of root-associated fungi that exhibit an independent origin of complex multicellularity and fruiting-body formation among basal ascomycetes. Taphrinomycotina, which also includes fission yeast (*Schizosaccharomyces*) and lung pathogenic *Pneumocystis* yeast, constitutes an ecologically diverse group where the majority of described species are dimorphic plant pathogens that belong to the Taphrinales order. Taking into account undescribed Taphrinomycotina diversity, Archaeorhizomycetes stand out as exceptionally diverse and is likely the most species rich lineage within the subphylum.

Material and methods: We sequenced the genomes of *A. finlayi* and *A. borealis* with PacBio and reconstructed a phylogeny including all the

main subphyla of Ascomycota. We performed comparative genomics across Taphrinomycotina and inferred changes in gene family size with CAFE. With BLAST, we identified genes related to sexual reproduction and estimated the number of rDNA copies per genome.

Results: We present the genomes of *A. borealis* and *A. finlayi* and thus contribute the first genomic data for Archaeorhizomycetes, which despite its prevalence in soil, has proven recalcitrant to culturing. We confirm the placement of Archaeorhizomycetes in Taphrinomycotina as a sister lineage to *Neolecta* with strong support. For comparative genomics, we focused on genes that are known to play important roles in fungal-plant interactions, namely: small secreted proteins (SSPs), peptidases, and carbohydrate-active enzymes (CAZymes). We found that Archaeorhizomycetes have the genes needed for sexual reproduction, despite no observed sexual structures. However, the precise sexual system of Archaeorhizomycetes is difficult to address since we can only identify one MAT locus idiomorph with certainty. For biological and technical reasons, abundance of fungal biomass cannot be directly inferred from relative read abundance in metabarcoding data. For example, rDNA copy number varies extensively across the fungal kingdom. We estimate the rDNA copies per genome to be 4 in *A. finlayi* and 10 in *A. borealis*, which is relatively low compared to the ascomycete average of 50 copies. This in turn indicates that the high read abundance of Archaeorhizomycetes in soil reflects real cellular abundance.

Conclusions: We confirm the placement of Archaeorhizomycetes in Taphrinomycotina with *Neolecta* as a sister lineage. Our results from comparative genomics analysis, rDNA copy number estimates, and mating system characterization, brings us towards a better understanding of the ecology and evolution of Archaeorhizomycetes.



Comparative analysis of Genes encoding Ribosomal Proteins across Metazoa

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POSTER SESSION 3 - **P03.087**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Protein synthesis (translation) is a necessary attribute of life as we know it. It is mediated by the translational machinery, in which ribosomes play a central role. Ribosomes are complexes of structural ribosomal RNA (rRNA) and proteins (e.g., ribosomal proteins, translation factors, tRNA synthetases) that universally share a core structure within their 2-subunits arrangement. Part of the genes encoding the translation machinery are shared as orthologues across the whole Tree of Life, and, as such, these genes have always been considered ideal as phylogenetic markers to investigate splits that happened in deep

time. However, when it comes to animals (Metazoa), a comprehensive characterisation of such genes –quantifying the number of their duplications, as well as the rate of evolution, for example– is still lacking. Here, to fill this gap, we analyse 136 gene families of translational genes across Metazoa. We present and discuss the distribution of such genes and the phylogenetic patterns across animals, as well as characterise some of their evolutionary features. This work aims to provide critical insights into the evolutionary dynamics of genes involved in translation, primarily Ribosomal Protein coding genes.



Comparative genomics provides insights into early angiosperm evolution

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POSTER SESSION 3 - **P03.071**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Angiosperms emerged through an early evolutionary burst over 150 million years ago, leading to the two major groups, the monocots and eudicots. These comprise over 95% of flowering plant diversity, but have key differences in their morphology, ecology and life histories. Pinpointing how these two groups evolved to be so different requires a detailed reconstruction of the genomic changes during flowering plant evolution. However, the macroevolutionary trends governing flowering plant gene family repertoire remain poorly understood.

Here by analysing 273 archaeplastid genomes representing 50 flowering plant orders, we identify high rates of gene loss and

duplication in the evolutionary history of angiosperms. Monocot diversification was accompanied by a period of reductive genome evolution while the first eudicot genomes experienced modest rates of gene duplication. Orders were characterised by rampant gene loss, compensated by modest gene duplication. This suggests that angiosperms reached a core genomic diversity early in their evolutionary history, corresponding to their high morphological diversity. This work highlights the importance of loss as well as gain of function in the diversification of the most speciose plant group.



Comparative genomics provides insights into early bryophyte evolution

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POSTER SESSION 3 - **P03.069**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Land plants originated from a streptophyte algal ancestor ~500 million years ago, marking one of the most significant events in Earth's history. On land, these ancient plants diverged into two major lineages: the monophyletic bryophytes and vascular plants. Recent findings have shown that the first bryophytes emerged through reductive evolution, implying they derive from a more complex ancestor. The main bryophyte lineages, hornworts, liverworts and mosses, display a patchwork of traits, many shared with vascular plants. This raises the question: does bryophyte trait distribution represent convergent or reductive evolution? And does this extend to their genes and genomes?

Here, I will present my work investigating gene content evolution accompanying the early diversification of bryophytes. This analysis, using sophisticated gene-tree-species-tree reconciliation methods, shows that hornworts, the first splitting lineage of bryophytes, have the largest occurrence of gene loss. Functionally, many of these lost genes are linked to the development of water conducting cells, a trait lost in hornworts. Further analysis demonstrates convergent evolution between genetic networks in vascular plants and water-conducting mosses. These analyses suggest that a patchwork of genetic mechanisms regulated early bryophyte evolution. This work is revealing the complexities of the earliest land plants, with implications for bryophyte and embryophyte trait evolution.



Convergent evolution of cosexuality in liverworts

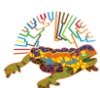
Szoevenyi P¹, Yasui Y, Potente G, Shimokawa E, Jenkins J, Walstead R, Grimwood J, Schmutz J, Leebens-Mack J, Bruna T, Kaur N, Lee R, Zama S, Tanaka T, Umeya Y, Kawamura S, Yamato K, Shigenobu S, Shimamura M, Kohchi T

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POSTER SESSION 3 - P03.075, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Sex chromosomes are expected to coevolve with their respective sex, potentially disfavoring their co-occurrence as cosexuality evolves. This effect is expected to be stronger where sex chromosomes are restricted to one sex, such as in plants expressing sex in their haploid stage. We assess this hypothesis in liverworts with U/V sex chromosomes, ancestral dioicy, and several independent transitions to monoicy (cosexuality). We report the chromosome-level genome assembly of *Marchantia quadrata*, which recently evolved monoicy, and perform comparative genomic analyses

with its dioicous relative *M. polymorpha*. We find that monoicy evolved via retention of the V chromosome as a small ninth chromosome, complete loss of the U chromosome, and translocation of key U-linked genes to autosomes, among which the major sex-determining gene (*Feminizer*) acquired environmental/developmental regulation. Our findings parallel recent observations on *Ricciocarpos natans*, which evolved monoicy independently, suggesting genetic constraints that may make transitions to monoicy predictable in liverworts.



Does Metabolite Toxicity Impact Gene Order in Metabolic Operons through Selection for Robust Gene Expression?

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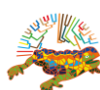
POSTER SESSION 3 - **P03.070**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Bacterial genomes are organized in operons, grouping multiple genes that are expressed in similar biological conditions. We study the order of genes within operons, a question that has received little attention. The specific question we ask is whether two genes catalyzing two successive reactions of a metabolic pathway are encoded in the same order within their operon than they appear within the pathway. A pioneer study has shown 15 years ago that the answer is yes for more than half of such gene pairs, and suggested a mechanism leading to a selection pressure for coding genes in this order. On the opposite, in this work we are looking for selection pressures which could explain why a significant amount of gene pairs are encoded in the invert genetic order.

For all metabolic operons of *E. coli*, we computed several features which could directly or indirectly reflect the benefit of the invert genetic order, such as essentiality of each gene, fitness defect associated with

knockout or over-expression of each gene, average expression level of the operon, potential toxicity of the intermediate metabolite between the two reactions, and conservation of the genes in their order in other organisms.

We found that fitness defect caused by knockout of the downstream gene within the pathway is significantly associated with the inverse gene order (in which this downstream gene is encoded first in the operon), suggesting that this inverse order arises from a selection pressure for strong and robust expression of the downstream gene. We postulate that in some pathways, this selection pressure could be caused by the toxicity of the intermediate metabolite, leading to a benefit in stronger and more robust expression of the gene which consumes the metabolite than the gene which produces it.



Evolution of amylase copy number variation in Nile rats and functional impact

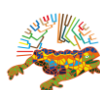
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POSTER SESSION 3 - **P03.080**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The Nile rat (*Arvicanthis niloticus*) is a valuable diurnal model for studying metabolic diseases due to its human-like circadian rhythm and strong susceptibility to diet-induced diabetes, particularly on starch-rich diets. Evolutionarily, Nile rats may have lost their ancestral ability to efficiently metabolize starch, as their native diet consists primarily of fiber-rich, starch-poor vegetation. Amylase is a key enzyme for starch digestion, and its encoding gene (AMY) exhibits copy number variation (CNV) across mammals, often correlating with dietary starch consumption. In humans, AMY CNV has been associated with metabolic traits such as obesity and diabetes, and is under selection in starch-consuming populations. We hypothesized that the amylase gene cluster evolved uniquely in Nile rats as part of an adaptive response to their natural diet, with functional consequences for starch metabolism and disease susceptibility. To explore this, we employed three complementary approaches. First, we genotyped AMY CNV using droplet digital PCR (ddPCR) in 159 Nile rats and observed copy numbers ranging from 4 to 12 per diploid genome. Notably, individuals with higher AMY copy numbers were significantly more likely to develop diabetes on a high-starch diet ($p < 0.01$), suggesting a gene dosage effect on metabolic disease risk. Second,

transcriptome analyses across multiple tissues revealed that Amy2 and a recently duplicated paralog, Amy2', are specifically expressed in the pancreas, while Amy1 is expressed in the parotid salivary glands. This tissue-specific expression supports subfunctionalization of the amylase paralogs. Phylogenetic reconstruction indicated that Amy2' is a species-specific duplication within Nile rats, while Amy1 likely originated from an older duplication event preceding the divergence of rats and mice. Third, to better understand the structural evolution of the AMY locus, we generated long-read, high-accuracy sequencing data from multiple Nile rat individuals. These data enabled full haplotype reconstruction of the amylase region and revealed extensive structural rearrangements, providing a clearer view of the CNV architecture. Together, our results uncover substantial amylase CNV in Nile rats, highlight its association with starch-induced diabetes, and reveal evolutionary and functional divergence among AMY paralogs. These findings offer insight into how gene duplication and structural variation can contribute to metabolic adaptation and disease, and they establish a framework for further investigation into the evolutionary genomics of diet-related traits in this emerging model organism.



Evolutionary diversity of CXCL16-CXCR6: Convergent substitutions and recurrent gene loss in sauropsids

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POSTER SESSION 3 - **P03.067**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: The interaction of chemokines with chemokine receptors guides immune cells to target sites, impacting immune defence efficacy. The CXCL16-CXCR6 axis is crucial for regulating CD8 tissue-resident memory T cells (TRM) persistence. CXCR6 serves as the core TRM marker gene along with CD69, ITGAE, and ITGA1. TRM cells are critical for effective immunity against pathogenic infections such as influenza. In mice, CXCR6 deficiency lowers TRM cell numbers in the lungs and depletes ILC3s in the lamina propria, impairing mucosal defence. This axis is linked to diseases like HIV/SIV, cancer, and COVID-19. The unique DRF motif of CXCR6 facilitates leukocyte adhesion by interacting with cell surface-expressed CXCL16 and plays a key role in G-protein selectivity during receptor signalling. Together, these highlight that the CXCL16-CXCR6 axis is pivotal in host immunity. Previous studies of the CXCL16-CXCR6 axis found genetic variation among species but were limited to primates and rodents.

Materials and methods: To investigate the evolutionary trajectory of CXCR6 and its ligand CXCL16, we performed a comparative genomic analysis encompassing approximately 425 vertebrate species, including fishes, amphibians, reptiles, birds, and mammals. We analysed the genome assemblies and short-read sequencing data using tools such as TOGA and BLAST to confirm gene loss. The G-protein coupling probabilities of CXCR6 were predicted using PRECOGx, while molecular dynamics (MD) simulations were employed to identify key interacting residues. To evaluate the functional implications of gene loss, we analysed publicly available single-cell transcriptomic datasets from mallard, pigeon, and chicken lungs. Furthermore, these genes' selection

signatures and convergent substitutions were analysed using Hyphy, PAML, and CSUBST.

Results: Our evolutionary trace analysis of the DRF motif found that this motif is not universal across all vertebrates. The DRF motif is restricted to mammals, turtles, and frogs, while the DRY motif, typical in other chemokine receptors, is found in snakes and lizards. Most birds exhibit the DRL motif. Furthermore, by using molecular dynamics simulations and the machine learning-based tool PREGOCx, we show that these substitutions at the DRF motif affect the receptor—Gi/o protein interaction. We establish recurrent CXCR6 gene loss in 10 out of 36 bird orders, including Galliformes and Passeriformes, Crocodilia, and Elapidae, attributed to segmental deletions and/or frame-disrupting changes. Notably, single-cell RNA sequencing of the lung shows a drop in TRM cells in species with CXCR6 loss. The reduced TRM cell abundance in species which have lost the CXCR6 gene further emphasises its importance in immune cell localisation and function. Furthermore, the concurrent loss of ITGAE, CXCL16, and CXCR6 in chickens may have altered CD8 TRM cell abundance, with implications for immunity against viral diseases and vaccines inducing CD8 TRM cells.

Conclusions: Our comparative genomic study for CXCR6 gene presence/absence establishes recurrent loss of the CXCR6 gene in sauropsid lineages via segmental deletion and/or frame-disrupting changes. Our results highlight the evolution and diversification of CXCL16-CXCR6 in sauropsids through the repeated concurrent loss of CXCL16-CXCR6 and variation at the DRF motif (DRF/DRL/DRY). Furthermore, species with CXCR6 gene loss may be targeted for therapeutic approaches to enhance TRM retention and improve vaccination outcomes.



Exploring the transcriptional landscape of environmental samples using metatranscriptomics long reads

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POSTER SESSION 3 - **P03.074**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Introns and splicing in eukaryotic marine organisms exhibit unique characteristics compared to other eukaryotes. In particular, splice sites in these organisms often do not adhere strictly to the GT-AG conventional rule, and we can find one or both of these terminal dinucleotides changed, resulting in what are termed non-canonical introns (NCI) (1–3).

During TARA Oceans (2009-2013), and later the TARA Pacific expedition (2016-2018) sea-water samples containing viruses, bacteria and plankton were collected (4,5), and some of them were sequenced using long-reads sequencing. These data represent a valuable resource to study non-canonical splicing in planktonic eukaryotes.

After adjusting aligning parameters to enable the detection of novel non-canonical splice sites, metatranscriptomic long reads were mapped to metagenomic data from TARA Ocean and TARA Pacific expedition, in order to extract exon-intron structures. This resulted in the identification of a variety of non-canonical introns, some of them likely corresponding to introner elements, i.e. introns derived from the insertion of transposon like sequences (6).

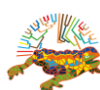
In total, a large number of introns were identified using several metagenomic references. After filtering and analysing them, we observed that some of them exhibited unique splice sites, revealing diverse and non-canonical splice sites within these environmental samples.

The results highlight the complex genetic diversity present in marine organisms and emphasize the importance of studying the underexplored marine environment, as it represents a significant source of novel genetic information. Research into non-

canonical introns will improve our understanding of evolution in marine ecosystems, contributing valuable knowledge to the study of marine biodiversity.

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Genome of the Amazon Guppy (*Poecilia bifurca*) reveals conservation of sex chromosomes and dosage compensation

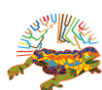
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POSTER SESSION 3 - **P03.077**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The Amazon guppy, *Poecilia bifurca*, is a small live-bearing fish. Related species, such as *P. reticulata*, *P. picta* and *P. parae* all share the same sex chromosome system, but with substantial diversity in the degree of Y degeneration and the extent of X chromosome dosage compensation. We built a female (XX) draft genome with 55X coverage of PacBio HiFi data, resulting in a 785 Mb assembly with 94.4% BUSCO

completeness. We used this genome to determine that *P. bifurca* shares the same sex chromosomes as related species, and that it exhibits substantial Y degeneration. We combined this with RNA-Seq data to reveal that *P. bifurca* also exhibits complete X chromosome dosage compensation. We further identify 11 putative autosome-to-Y gene duplications, five of which show gene expression in guppy male germ cells.



Lineage-specific selection and gene family dynamics in the genus *Lynx*

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POSTER SESSION 3 - P03.083, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: The investigation of species divergence and associated adaptations has remained a fundamental focus of evolutionary studies. Among mammals, the Felidae family is characterized by a rapid radiation, and a notable history of hybridization events among species. Within this felid family, the lynxes stand out as a globally distributed genus, comprising four species characterized by distinct ecological traits. In this study, we investigate the adaptive evolution in the *Lynx* lineage by integrating analyses of positive selection in protein-coding genes and gene family evolution dynamics.

Materials and methods: We leveraged recent high-quality genome assemblies for all four *Lynx* species to investigate patterns of adaptive evolution. To detect signals of positive selection, we applied codon substitution models to both the *Lynx* genus branch and each extant species-specific branch. From the Felidae phylome—comprising 14,738 coding sequences across 16 felid species—we identified 9,290 one-to-one orthologous genes. Positive selection was inferred using the branch-site model in codeml (PAML), considering only genes with significant support for both site and branch effects (BEB > 0.99 and LRT p-value < 0.05). These candidate genes were then subjected to Gene Ontology enrichment analysis using the topGO R package to identify overrepresented biological processes. In parallel, we explored gene family evolution using Count, analyzing a dataset of 13500 gene families. Gene family expansions, contractions, gains, and losses were inferred under a maximum likelihood birth–death model, and only lineage-specific changes with a posterior probability greater than 0.9 were retained.

Results: We identified lineage-specific signals of adaptation. In the ancestral *Lynx* genus

branch, positively selected genes were significantly enriched in neurodevelopment and intracellular signaling, suggesting adaptations in sensory and regulatory pathways. In *L. canadensis*, enriched functions related to metabolic regulation, insulin response, and neurotransmitter signaling, consistent with adaptations to extreme seasonality and energy management in cold climates. *L. pardinus*, the most endangered and geographically restricted lynx, showed signatures of selection in genes associated with development, reproduction, and immunity—likely reflecting demographic history and ecological constraints. In *L. lynx* and *L. rufus*, enrichment was observed in cellular communication, metabolism, and environmental response, paralleling their broad distributions and diverse ecological niches. We detected a total of 327 gene family expansions, 561 gains, 51 losses, and 168 reductions on the ancestral *Lynx* genus branch. These extensive changes suggest a substantial remodeling of the gene repertoire early in the evolution of the genus, potentially contributing to foundational adaptations preceding species diversification. While the functional repertoire of these gene families remain to be characterized, the magnitude and diversity of events indicate a dynamic phase of genomic innovation and streamlining during early *Lynx* evolution.

Conclusions: By combining coding sequence evolution and gene family dynamics, we identified molecular pathways potentially involved in both inter- and intraspecific divergence. These findings not only offer insights into lynx adaptation and speciation but also enhance our understanding of the broader evolutionary patterns within Felidae.



OrthoFinder: scalable phylogenetic orthology inference for comparative genomics

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POSTER SESSION 3 - **P03.076**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Inferring the phylogenetic relationship of genes is fundamental to contemporary biology research. By understanding these orthologous relationships, we can compare the genomes and evolutionary history of different species, and transfer knowledge between them. Many methods exist for inferring orthology, but all lack the scalability required to handle thousands of genomes. This is already a problem now, and the challenge is increasing as multiple sequencing projects continue to expand the landscape of available genomes. We have previously developed OrthoFinder, a tool that uses a phylogenetic approach to identify orthogroups – a set of genes descended from a single gene in the last common ancestor of the species in question. OrthoFinder exhibits high accuracy in benchmarking analyses, but previously lacked scalability to more than a few hundred species. Here, we present a major advance to OrthoFinder that significantly improves both scalability and accuracy of the method, enabling orthology

inference on thousands of species. OrthoFinder achieves these improvements using a new approach of first inferring core orthogroups for a subset of species, and then assigning new genes to these orthogroups. This approach therefore avoids the computationally costly all-versus-all sequence search. We show that OrthoFinder is more scalable than other methods, being the only method able to infer orthology on 512 eukaryote genomes in less than 2 days. Importantly, this increase in scalability does not come at a cost to accuracy, as OrthoFinder outperforms all other methods in orthogroup and ortholog benchmarking. OrthoFinder is easy to use, and provides many useful outputs for users, including orthogroup gene trees, analysis of gene duplications, and extensive comparative genomics statistics. OrthoFinder is available on conda, and full instructions, source code, and tutorials are available at <https://github.com/OrthoFinder/OrthoFinder>.



Studying the evolution of gene repertoire in Fisher's Geometric Model

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POSTER SESSION 3 - **P03.072**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Fisher's Geometric Model (FGM) is a simple, yet powerful, model of evolution. It has been widely used to study the effect of complexity (viewed as the number of traits under selection) on phenotypic evolution. However, because it describes mutations in terms of their phenotypic effect, it cannot be used to study evolution at the genetic level. We propose a simple reinterpretation of the FGM that allows to study the dynamics of gene duplication/deletion and the evolution of the genetic repertoire. Our model conserves the geometric representation of the FGM but, while in the canonical FGM mutations directly displace the phenotype in an n-dimensional phenotypic space, we add a genetic layer that behave as "phenotypic-modifier": starting from a given phenotype (represented as a point in the phenotypic space), each gene is an n-dimensional vector that modify the phenotype by a simple addition. This simple reinterpretation enables to model the evolution of an individual by a mixed process of mutations (that randomly modify the gene vectors), gene duplications (that duplicate a random gene vector) and gene loss (that delete a random gene vector). Other mutational processes can also be introduced (e.g., whole-genome duplication, de novo gene origination or horizontal gene transfer) but have not been studied so far.

We used this reinterpretation to study the relative advantage of the different types of variation (gene mutation, gene duplication and gene loss) depending on the phenotypic complexity and on the distance to the optimum. Unsurprisingly, results show that gene duplication is more advantageous than gene mutation when far from the optimum, mutations being in turn more advantageous when close to the optimum. But the model also exhibit more surprising behaviors. In

particular, it shows that the fate of duplicated genes could be more complex than naively expected. Indeed, a duplicated gene, initially favorable, could later becomes deleterious due to the evolution of its genomic context. This shows that gene loss can be advantageous, even in situations where the initial duplication of this same gene had been advantageous, illustrating the power of this new model in representing complex epistatic patterns. Finally, while the canonical FGM is well known for its results on phenotypic complexity, our reinterpretation enable to study the effect of genetic complexity. When starting far from the optimum, the dynamics of evolution can roughly be divided into two phases. First the growth phase during which the genetic repertoire of the organism expands, resulting in rapid fitness gains. Second a plateau phase where the phenotype is so close to the optimum that all duplications are deleterious and the organism must rely on point mutations to approach the optimum. However, duplications, which increase the genomic complexity of an organism, have antagonistic consequences on evolution in the long run: at constant phenotypic complexity organisms that have accumulated genes in the first phase end up with a much lower fitness than simpler organisms that have only evolved through point mutations. This demonstrates the existence of a cost for genetic complexity that is superimposed on the classic cost for phenotypic complexity.

We think that this reinterpretation of the FGM can serve as a basis to mathematically study the evolutionary dynamics of the gene repertoire under the mixed pressure of mutations, gene duplication, and gene loss.



The genome distribution of structural mutations in wild strains of *Neurospora crassa*

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POSTER SESSION 3 - **P03.086**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Identifying structural mutations (SM), also known as structural variants, is to identify genetic biological diversity. Research into mutation rates and evolution of mutagenesis have long been restricted to point mutations due methodological limitations. Identifying SMs is a challenging task as both sequencing and computational methodology have different biases when identifying SMs. Here, we make use of Oxford Nanopore long reads in addition to Illumina short reads as complementary verification when we made de novo assemblies of 53 wild strains of the fungal model species *Neurospora crassa*. Furthermore, by applying multiple computational mapping models, we have identified and cross-checked the resulting SMs and have analysed their distribution across the genome.

First, we compared single nucleotide polymorphisms (SNPs) and SMs by inference of nucleotide diversity as by Watterson's theta. We find that SNPs and SMs correlate poorly and follow different distribution patterns along the chromosomes. Our results suggest that the mutagenic processes underlying the distribution of SNPs, are significantly different from what causes the prevalence of SMs.

Furthermore, we are specifically interested in the differences between heterochromatic and euchromatic regions. Heterochromatic

regions are tightly packed, whereas euchromatin is more loose, accessible for transcription, and harbours the majority of protein coding genes. In the species *N. crassa*, most of the genome is euchromatic and there is little non-coding DNA. Considering sequence length of the regions, we find that the distribution of SMs differ between heterochromatin and euchromatin, but also that the ratio differs between the different kinds of heterochromatin; centromeres are the most enriched for SMs, whereas euchromatin is not. Additionally, assuming that SM are deleterious, we found more SMs in genic regions than we expected. However, we find that when a SM overlap with genes, they are often found in introns, and this applies also to SMs in genes found in heterochromatic regions.

Taken together, we find that SMs are enriched in locations that appear non-detrimental. However, the uneven distribution between heterochromatic regions also suggest the regions undergo differing selective pressure with respect to mutations. We will elaborate on the distribution of SMs in genic regions and discuss the implications for both gene and genome evolution, and what the differing distribution may tell us of previously unannotated genomic sequences.



Thymelaeaceae genome annotations: where are we?

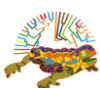
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*POSTER SESSION 3 - **P03.084**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM*

With increasing numbers of published genome sequences, annotations of protein coding genes proliferate. Producing and evaluating these annotations can be hard, in particular in the face of problematic annotations being used as a basis for subsequent ones, both within and across species. This poster provides a closer look at

a number of genome annotations for species belonging to the Thymelaeaceae family, evaluating them and contrasting the methods used to generate them. Strengths and potential weaknesses are highlighted, and common pitfalls are discussed along with recommendations for quality assurance.



Uniform annotations reveal the connections between genome size, repeat content and gene family evolution

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POSTER SESSION 3 - **P03.081**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Genome size (GS) is a fundamental biological characteristic and its evolutionary causes and consequences are topics of ongoing debate. While GS correlation with repeat content has been shown in several taxa, associations with other characteristics such as gene number remain unclear, and understanding how these characteristics interact with GS variation across species can be difficult when data comes from multiple sources. Here we study GS evolution in Coleoptera for the first time from the perspective of gene family and repeat content evolution using whole genome annotations to estimate the number of genes in the genome. We chose 14 species across the suborder Polyphaga with existing annotations that span over 200 million years of evolution, four of which have genome size measurements from flow cytometry. These measurements are very similar to the assembly sizes, which we therefore use as a proxy for genome size in this work. We ask how the genic and repeat content varies across the species in relation to GS variation, and whether GS is associated with genome-wide birth-death dynamics.

We first demonstrate the impact of heterogeneous computational pipelines on gene prediction by comparing native genome annotations available in published databases to uniform annotations across 14 species that we have generated. Past research implies and our results confirm that a difference in both computational pipeline and included reference data can lead to sizeable differences in the resulting protein sets, which is why we have decided to unify our pipeline as much as possible by excluding species-specific RNA evidence data. When comparing the different annotations for our species, we find that the proportion of non-overlapping exons stays relatively constant, but consistently grouping

exons into the same transcripts proves to be more difficult for larger, repeat-rich genomes. Here, we also show that adding a post-processing step for the protein set to assess and filter repeat contamination is important even for masked assemblies when repeat content is high. In addition, the repeat distribution across the genome is quite different even for closely related species, with centromeres especially showing interesting variation.

We then test whether GS and repeat content are associated with significant changes in gene family sizes across species. We use the well-established workflow of computing orthogroups with orthofinder and then identifying rapidly evolving orthogroups with CAFE5, where we compare the native annotation with our above-described uniform pipeline. We show that there are large fluctuations in the number of individual genes between the species, and the annotation pipeline affects how well such variation can be estimated. However, the number of gene families is conserved for both annotation methods. The significantly rapidly evolving orthogroups are contributing greatly to the increased number of genes in many species, and their size is significantly associated with the genome size for the uniformly annotated dataset, while showing no significant association with repeat content.

Finally, we discuss the best practices for genome annotations for comparative genomics in relation to the challenges that arise from GS variation. This study advances our understanding of genome evolution in Coleoptera, especially concerning how it is related to both the number of genes and the repeat content, and provides evidence that gene duplications proliferate in repeat-rich larger genomes.



Widespread intra- and inter-domain horizontal transfer and bursts of gene duplication shape the size and content of Asgard archaeal genomes

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POSTER SESSION 3 - P03.082, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Asgard archaea are sister to eukaryotes and thus pivotal in understanding eukaryogenesis. Early analyses of Asgard genomes uncovered larger sizes as compared to other archaea and the presence of many genes of putative bacterial origin. However, our knowledge on the relative impact of horizontal gene transfer and gene duplication in shaping Asgard genomes is still limited. Here we used phylogenomics of 31 representative Asgard genomes to understand the Asgard pangenome, assess gene duplication and loss, as well as inter- and intra-domain gene transfer. We found

that gene transfer is pervasive throughout asgard evolution, involving mostly metabolic genes that are peripheral in interaction networks. However, we found that transfer levels are not higher than those in closely related prokaryotes, and that gene duplications, rather than transfers, underlie increased genome sizes in Asgard archaea. Hence, consistent with their phylogenetic position, asgard genomes showcase a unique blend of prokaryotic-like transfer-driven and eukaryotic-like duplication-driven evolution.



Boom and Bust in the Parasite Genome: The adaptive benefits of massive gene family losses in slave-maker ants

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MEETING ROOM 118+119, August 22, 2025, 10:30 AM - 12:30 PM

Background: The evolution of gene families is often viewed through the lens of family growth by gene duplication and subfunctionalisation with an implicit assumption that this process is adaptive and largely irreversible. However, the evolution of an obligate parasitic lifestyle often leads to the reduction of morphological and physiological traits, which may be accompanied by loss of genes and functions. We studied both process in the iconic system of pairs between slave-maker ants and their hosts. This system has puzzled many researchers since Darwin because it is akin to the reversal of the evolution of sociality which in turn is considered a major transition in evolution. Slave-making ants are social parasites that manipulate their hosts and exploit the work force of closely related ant species for social behaviours such as brood care and foraging. Recent divergence between these social parasites and their hosts allows accurate comparative studies of gene family evolution.

Methods: We sequenced the genomes of 15 ant species, representing four independent origins of ant slavery and several outgroup. During the evolution of eusociality, chemoreceptor genes multiplied due to the

importance of chemical communication in insect societies.

Results: We investigated the evolutionary fate of these chemoreceptors and found that slave-making ant genomes harbored only half as many gustatory receptors as their hosts, potentially mirroring the outsourcing of foraging tasks to host workers. In addition, parasites had fewer odorant receptors and their loss shows striking patterns of convergence across independent origins of parasitism. Losses affected in particular orthologs which are often implicated in sociality like the 9-exon odorant receptors, particularly those associated with the evolution of sociality. Hosts, however, retain many duplicated genes.

Conclusions: These convergent losses represent a rare case of convergent molecular evolution at the level of individual genes. Thus, evolution can operate in a way that is both repeatable and reversible when independent ant lineages lose important social traits during the transition to a parasitic lifestyle. Our findings suggest that while hosts primarily rely on existing molecular mechanisms for defence, parasite genome evolution is characterised by extensive, convergent gene loss, innovation, and network rewiring.



Evolution of genome size in self-fertilising populations

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MEETING ROOM 118+119, August 22, 2025, 2:00 PM - 3:00 PM

Genome size is subject to rapid change on the evolutionary timescale. Verbal and mathematical models predict that selection could act to reduce the total genome size to avoid unnecessary metabolic costs. Generally, the role of effective population size is considered central to understanding the dynamics of genome size. Larger effective population size is expected to yield better selection for streamlined and smaller genomes. Small effective population size, implying less effective selection and stronger genetic drift, is expected to result in larger genome sizes. However, the evolution of self-fertilisation, a reproductive mode known to drastically decrease the effective populations size, is correlated with a notable decrease in genome size. The reason for this is not quite understood, though it has been strongly suggested that it is due to the decrease in the number of Transposable Elements. Though these parasitic genomic elements do contribute to genome size, they

are not enough to explain the observed differences in genome size between self-fertilising and outcrossing species. Here we present simulation results using the eukaryote extension of the Aevol digital genetics platform to examine how self-fertilisation influences genome size and content. We find that, even in the absence of Transposable Elements, there is ongoing selection for smaller genome sizes in self-fertilising populations, despite their smaller relative effective population sizes compared to outcrossing populations. This is mainly due to a reduction in non-coding DNA, which agrees with empirical evidence in *Arabidopsis*. Possible explanations for this are that smaller genome size allows a decrease in the mutation rate, and more streamlined genomes reduce the probability of illegitimate recombination events, resulting in, on average, better offspring fitness.



Genome Size Reduction Under Non-Adaptive Evolution in an Island Radiation

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MEETING ROOM 118+119, August 22, 2025, 2:00 PM - 3:00 PM

Background: Oceanic islands, with their geographic isolation and well-characterized geological histories, offer a powerful natural framework for studying the evolutionary processes that have shaped current patterns of biodiversity. After colonizing the Canary Islands, the red devil spiders of the genus *Dysdera* underwent an extraordinary diversification, largely associated with trophic specialization. Remarkably, the genomes of these island endemic species are nearly half the size of their mainland counterparts (~1.7 vs. ~3.3 Gb). This provides a unique model system to address long-standing questions about the relative contributions of adaptive and non-adaptive processes in the evolution of genome size in invertebrates.

Materials and methods: Here, we addressed this question by conducting a comprehensive comparative genomic analysis using three high-quality chromosome-level assemblies, two from island species and one from mainland species, two of which were generated specifically for this study. This approach allowed us to dissect the contribution of different genomic elements, including genes, structural variants, repetitive elements and multigene families, to the observed change in genome size.

Results: Contrary to expectations, we found that insular species exhibited a widespread reduction in genome size, impacting all genomic elements, including intronic and intergenic regions, with transposable element (TE) loss accounting for most of this contraction. Besides, autosomes experienced a disproportionate DNA loss compared to the X chromosome. Paradoxically, we also found that island species exhibit higher levels of nucleotide diversity and recombination, lower TE activity in recent times, and evidence of intensified natural selection, compared to their mainland counterparts, consistently supporting larger long-term effective population sizes in the Canary Island species.

Conclusions: Overall, our findings align with the non-adaptive mutational hazard hypothesis, in which purifying selection against slightly deleterious DNA and TE insertions -favoured by high long-term historical effective population sizes- was the primary mechanism driving genome size reduction in island species. This study offers new insights into the evolutionary forces and molecular mechanisms shaping species diversification on oceanic islands and invites us to reconsider classical expectations about the evolutionary dynamics underlying adaptive radiations.



Natural protein structures have evolved exceptional robustness to mutations

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MEETING ROOM 118+119, August 22, 2025, 10:30 AM - 12:30 PM

Conservation of protein structure between highly divergent sequences is well-established, yet how it arises from the evolutionary dynamics of protein folding is unclear. One important source of insight has come from the study of RNA structures, where a broad spectrum of mutational effects renders molecules robust as well as evolvable. Thanks to the recent revolution in protein structure prediction, similar systematic analyses are now possible for proteins. We find that most natural protein structures are highly robust to point mutations but lack the structural variation in mutants that is key for evolvability. Whereas the robustness (mutational profile) of functional RNA sequences resembles that of

random RNA sequences, this is not the case for established proteins. Interestingly, the mutational profile of many de novo proteins does resemble random amino acid sequences, featuring low robustness. Thus, high mutational robustness is a rare and evolved property of established proteins. This striking finding has major implications for our understanding of the evolution of genomes, for example by providing an explanation for the prevalent reuse of existing and robust folds for novel functionalities via gene duplication rather than the discovery of novel structures for those functionalities through de novo gene birth.



The march to land: unearthing how gene repertoire evolution triggered terrestrialization across the Animal Tree of Life

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MEETING ROOM 118+119, August 22, 2025, 10:30 AM - 12:30 PM

The transition from sea to land—terrestrialization—is one of the most extraordinary evolutionary events that has shaped Earth’s biodiversity. This monumental shift required animals to overcome environmental challenges and adapt to life on land through profound biological changes. Terrestrialization occurred independently in nine animal phyla, often with multiple transitions, offering a unique window into the genomic basis of extreme convergent evolution. Despite its evolutionary importance, the genomic underpinnings of terrestrialization across the animal kingdom remain largely uncharted. Through the lens of comparative phylogenomics and machine learning, we analyzed the gene repertoire evolutionary dynamics of ~1,000 high-quality metazoan genomes and transcriptomes (~24 million genes), spanning virtually all animal phyla. Our findings reveal that gene loss was ten times more prevalent than gene gain in independently terrestrialized lineages,

suggesting that adapting to the specific environmental pressures or constraints associated with life on land more often involved the reduction or removal of no longer advantageous genetic elements, rather than the acquisition of new genes. We also uncovered a dynamic ‘terrestrialization genetic toolkit’ formed by genes of ancient origin that were co-opted in parallel for key terrestrial functions, and the convergent emergence of new genes across different lineages. These adaptations included mechanisms for desiccation tolerance, structural support, odorant reception, DNA repair, xenobiotic processing, and nutrient acquisition. Our study provides unprecedented insights into the genomic foundations of terrestrialization, revealing how ancient genetic resources and convergent innovations have collectively shaped the remarkable evolutionary trajectories that allowed animals to conquer land.



Understanding Evolutionary Changes in the Genome through investigation of Evolutionary Breakpoint Regions and Homologous Synteny Blocks

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MEETING ROOM 118+119, August 22, 2025, 10:30 AM - 12:30 PM

Background: Understanding the evolutionary mechanisms driving speciation requires examining changes at the sequence, transcriptome, and regulatory levels across species. Vertebrate genomes can be divided into multi-species homologous synteny blocks (msHSBs)—genomic regions maintained collinear between multiple species—or evolutionary breakpoint regions (EBRs)—demarcating genomic rearrangements where synteny is lost. Previous research has shown that msHSBs tend to include functionally vital genes, whereas EBRs, in contrast have been associated with lineage-specific genomic features. However, these studies lack an integrative analysis of the several layers where evolutionary pressures might have taken place. Using previously defined EBRs, we explored their genomic, transcriptomic, and epigenomic contexts to understand the mechanisms behind their formation and their impact on species evolution.

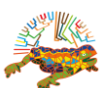
Materials and methods: At sequence level, we investigated gene density, transposable elements, and genes under positive selection. Using gene annotations of nine vertebrate species, we defined orthologous genes using Orthofinder. Then, genes under positive/negative selection were identified with codeML.

To assess evolutionary changes at gene expression level, we identified housekeeping genes with transcriptomic data across nine tissues across nine species. Genes with dynamic expression evolution were detected using CAGEE, and gene ontology enrichment and gene network analysis assessed their function. Spatial genome organisation was analysed using published GPSeq data, capturing radial

positioning of chromatin digestion sites within the nucleus. Permutation testing assessed the relationship between these features and EBRs and msHSBs.

Results: We identified 238 vertebrate, 305 mammalian, 165 placental mammal, and 49 primate ancestral housekeeping genes, supported by gene ontology enrichment analysis. Additionally, 1786 genes with dynamic expression were detected in humans, but only two in the primate ancestor. Overall, our results show that EBRs are in gene-dense regions, but they do not overlap with genes under negative selection or housekeeping genes. EBRs are predominantly found in close proximity to the nuclear centre, with a negative association to the nuclear lamina. In contrast, msHSBs are enriched in housekeeping genes, dynamically expressed genes, and genes under negative selection. Gene ontology analyses associated EBRs to adaptive processes and msHSBs to essential cellular functions.

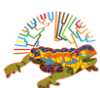
Conclusions: Our study provides significant insights into the relationship between specific genomic features, transcriptomic and regulatory trends and EBRs and msHSBs. We can subsequently infer distribution patterns and potential causes/consequences of genomic rearrangements. EBRs suggest a role of rearrangements in species-specific adaptations shown by their association with lineage-specific features and repetitive elements. However, while rearrangements disrupt synteny, they do not generally affect essential genes, gene expression or major regulatory processes, instead, these are enriched in msHSBs. Our results may provide an insight to the drivers of genome rearrangements and subsequently evolutionary changes.





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S26 - Gene flow to the rescue: Assessing the need, effectiveness, risks and ethical implications of manipulating gene flow to improve adaptation to climate change



Environment and forest management: How forest context drives gene flow and demographic dynamics in the rainforest timber tree *Dicorynia guianensis*

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POSTER SESSION 2 - **P02.157**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Understanding how ecological and human-mediated factors shape seed and pollen dispersal, spatial genetic structure (SGS), and demographic processes in tropical tree species is essential for their conservation and sustainable management. *Dicorynia guianensis*, a dominant canopy tree widely harvested in French Guiana, is known for its ecological and economic importance. Here, we combined nuclear and chloroplast microsatellite markers (SSRs) to investigate the genetic diversity, regional and fine-scale SGS, and dispersal patterns across multiple natural populations distributed along environmental and disturbance gradients. Spatial genetic structure was analysed on three life stages (juveniles, intermediates, and adult trees) allowing us to explore how ecological and demographic processes shape genetic structure across cohorts. Genetic diversity was generally high and remained stable across size classes, but patterns of inbreeding and SGS varied. Juvenile cohorts were more strongly structured and more inbred, likely reflecting limited dispersal and kin aggregation.

Intermediate and adult trees showed a progressive decrease in SGS, consistent with density-dependent thinning. Parentage analyses, complemented by cpSSR-based maternal assignment, allowed for the inference of effective seed dispersal. Seed and pollen dispersal distances varied across populations and cohorts, which could be linked to site-specific ecological conditions. We also detected seed dispersal events exceeding previously reported distances for *D. guianensis*, suggesting the potential role of undocumented secondary seed dispersal by vertebrates. Our findings demonstrate that stand demographic history and contemporary ecological and management-related processes, including stand density and disturbance regimes, shape genetic diversity and structure in *D. guianensis* populations. By integrating genetic, spatial, and demographic data, this study contributes to a more comprehensive understanding of reproductive ecology in tropical forests and offers valuable insights for the design of conservation strategies.



Evolutionary potential in a plant at the brink of extinction: *Centaurea corymbosa*

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POSTER SESSION 2 - **P02.156**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: *Centaurea corymbosa* (Asteraceae) is a narrow endemic plant species, occurring in the Massif de la Clape near Narbonne, in the South of France. There are only six known small populations located in an area of 3km², which makes it an important part of the natural heritage of France. The species is red-listed and threatened by short to mid-term extinction, as all populations are in decline, which is predicted to be aggravated by a higher frequency of drought in the future. There is very little contemporary gene flow between extant populations, which are highly differentiated. Conservation efforts have been ongoing to maintain natural populations of the species, among them a reinforcement of the natural populations with seeds produced in a common garden through free crosses between plants originating from different populations. The reinforcements thus introduced novel genetic variation within populations, equivalent to human-assisted gene flow. Can this introduction of genetic diversity rescue the *C. corymbosa* declining populations? Theory suggests that rescue is more likely if the reinforcements replenish genetic variation for life history traits that have a large impact on population growth. We want to evaluate how much additive genetic variation for different life history traits is present in seed lots used for reinforcements in *C. corymbosa*.

Material and methods: In a first step, seeds were collected in the 5 of the 6 natural populations and those seeds were grown in a common garden and produced the F1 generation. Those F1 seeds were used in the reinforcement protocol. A subset of these seeds were used in a classical common garden experiment to estimate additive genetic variances for different phenotypic traits, including survival, plant growth, phenology of flowering, capitula and seed production. Maternal sib families were planted in the garden and followed for two years until most plants flowered and died. We will genotype every plant in the garden with newly designed SSR markers to estimate relatedness between individuals and identify the population of origin of paternal plants.

Results: In this poster I will present a first analyses of phenotypic and between families variation and covariation for different life history traits in the experiment. I wish to discuss with colleagues at the meeting about alternative methods and packages to estimate genetic variances using genotyping information, as well as for testing for the effect of between populations crosses on the fitness of the F1.

The poster will also present how the results can be applied in the conservation of the natural populations of *Centaurea corymbosa*.



Genetic rescue from the brink of extinction: genomic consequences of a severe bottleneck and intense conservation management in the pink pigeon

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POSTER SESSION 2 - **P02.164**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The pink pigeon (*Nesoenas mayeri*) is an endemic bird of Mauritius that nearly went extinct in the 1990s, when the wild population declined to just ten individuals. Through extensive conservation efforts, including captive breeding, genetic rescue, and long-term supplementary feeding, the species has recovered to a population of around 450 individuals. However, this demographic rebound conceals ongoing genomic erosion and heightened susceptibility to emerging diseases, threatening the species' long-term viability and adaptive potential to environmental change. To understand the genomic consequences of the pink pigeon's near-extinction and subsequent recovery, we generated and analysed whole-genome data from 60 individuals spanning 180 years. Our sampling includes three key timepoints: (Historical) Ten museum specimens collected between 1830 and 1940, representing the genetic diversity present before the bottleneck. (Founders) 29 individuals sampled between 1990 and 1999, representing the period of the lowest population size. During this time the population received intensive genetic rescue with more than 200 individuals translocated from captive populations. (Modern) 21 contemporaneous individuals representing the recovered population that is suffering the consequences of inbreeding and exhibits susceptibility to trichomonas parasites. Using this temporal framework, we

examined genome-wide diversity, inbreeding, immunogenetic diversity, and genetic load trends. We also used demographic modelling to reconstruct changes in population size over time. Our results reveal a 56% loss of genetic diversity in the approximately six generations separating historical and founder individuals. This decline is accompanied by a rise in inbreeding coefficients, consistent with the sharp demographic decline during this period. Through the 1990s, genetic diversity continued to decline, though less steeply. However, the modern population exhibit a slight rebound of genetic diversity, demonstrating the positive impact of past genetic rescue. We show how rapid and sustained conservation actions played a key role in halting further genetic erosion. Nevertheless, diversity levels in the modern population remain significantly lower than those in historical samples, reflecting the lasting loss of genetic diversity and possibly its adaptive potential, leaving the species susceptible to emerging disease and environmental change. We discuss the risks and benefits of future genetic rescue, including extreme solutions like gene editing. We highlight how time-series genomic data can uniquely reveal patterns of genomic erosion and help evaluate the efficacy and limitations of genetic rescue efforts in endangered species.



Harnessing Genetic Rescue: A Case for Marsh Fritillary Butterflies in a Fragmented Habitat

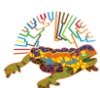
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POSTER SESSION 2 - P02.162, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Genetic rescue through assisted gene flow is a promising strategy to alleviate inbreeding depression and enhance genetic diversity in small, fragmented populations. Yet its broad-scale application is often hindered by concerns about outbreeding depression. Building on the widely used framework proposed by Frankham et al. (2011), we present a genomics-informed version that enables a more rigorous and transparent evaluation of genetic rescue feasibility. The updated framework integrates genomic insights on population structure, inbreeding, demographic history, and signals of selection to directly assess the key assumptions underlying outbreeding depression risk - namely, time since divergence, occurrence of recent gene flow, and the presence of local adaptation.

We demonstrate the utility of this framework through a case study of Danish marsh fritillary butterflies (*Euphydryas aurinia*), which show high inbreeding, shallow divergence, recent gene flow, and little evidence for local adaptation. These patterns suggest that assisted gene flow could be safely implemented even between the most genetically divergent populations with minimal risk of outbreeding depression. Our approach illustrates how meaningful conservation insights can be derived from genomic data even when working with scaffold-level assemblies and moderate sample sizes. By refining an established framework, this work contributes a generalizable model for integrating evolutionary genomic data into conservation planning under increasing habitat fragmentation and climate change.



Hybrid regeneration dynamics in a mixed plantation of interfertile species

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POSTER SESSION 2 - **P02.167**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The European silver fir (*Abies alba*) plays both economic and ecological roles in maintaining the functional balance of European forests. Its current distribution in Europe is the result of recolonization after the last glaciation contributing to local adaptations across Europe. Despite this adaptive capacity, signs of decline are observed particularly in France. As a response, assisted gene flow strategies have been proposed to enhance adaptive potential by introducing drought-resistant Mediterranean fir species. These species belong to the Euro-Mediterranean *Abies* clade and diverged during the late Miocene to early Pliocene (3.4–5 million years ago). Despite these divergences, the seven sister species are not reproductively isolated, as evidenced by artificial crosses and naturally occurring hybrids. In France, the most commonly planted Mediterranean fir species included *A. cephalonica*, *A. nordmanniana*, and *A. pinsapo*.

This study focuses on a 40-year-old plantation on Mont Ventoux (France, Vaucluse) composed of pure *A. cephalonica* and interspecific hybrids. A total of 272 adult trees were mapped and genotyped using 60 SNPs and 15 SSRseq markers. Natural regeneration was assessed in 1,500 seedlings spanning multiple cohorts from 1999 to 2017, all similarly mapped and genotyped. To identify the nature of the

seedlings, we performed clustering analyses using different types of approach (Multivariate analysis, Bayesian models, Frequentist models) thanks to the DAPC, STRUCTURE, and GENCLASS model. Additionally, parentage relationships were assessed with CERVUS and the R package “related”. Subsequently, GLM models were developed to analyze the relationships among species status within seedlings, as well as their survival, growth, and age structure.

The clustering results with STRUCTURE showed that 215 of the studied seedlings are pure *Abies cephalonica*, while 1,265 are considered hybrids. We identified 11 hybrid types, seven of which primarily involved *Abies cephalonica*. A GLM binomial survival model indicated that species status significantly explain seedling survival. *A. cephalonica* seedlings consistently had a lower chance of survival than the hybrids. Regarding growth, the GLM gamma model showed no significant effect of hybrid type on growth, but age played a significant role. Older seedlings exhibited greater growth than younger ones.

These preliminary results suggest that hybrids may exhibit higher survival rates compared to pure *Abies cephalonica* individuals, indicating a potential improvement in offspring fitness



Impact of Habitat Fragmentation on Genetic Health in European Hamster Populations: A Microsatellite and Genomic Analysis

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POSTER SESSION 2 - **P02.159**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Agricultural intensification across Europe has driven widespread habitat fragmentation and biodiversity loss. These changes are particularly detrimental to species with limited dispersal ability, such as the European common hamster (*Cricetus cricetus*), now critically endangered. In Hesse, Germany, one hamster population has been lost nearly every year since 1995, largely due to the isolation of remaining groups in small habitat patches. Understanding the genetic consequences of this fragmentation is vital for informed conservation planning.

We analyzed genetic diversity, structure, and inbreeding in *C. cricetus* using both microsatellites and whole-genome sequencing (WGS). A total of 841 individuals from six population groups—Core, Langgöns, Hochheim, Pohlheim, Extirpated, and Reference—were genotyped at 17 microsatellite loci. In addition, genomic data were generated for 12 individuals to assess fine-scale population structure and inbreeding patterns.

Microsatellite analysis revealed substantial polymorphism, with an average of 10 alleles per locus. The Core and Reference populations exhibited significantly higher allelic richness and observed heterozygosity compared to the satellite and extirpated groups. The Reference population harbored the highest number of private alleles, while the Extirpated and satellite populations showed reduced diversity.

STRUCTURE analysis based on microsatellite data indicated that at $K = 2$, Langgöns and Pohlheim clustered together, while the Core, Extirpated, and Hochheim populations formed a second group. At $K = 3$,

Pohlheim began to differentiate from Langgöns. Genomic STRUCTURE analysis using SNPs from 12 individuals identified $K = 2$ as the most likely number of clusters, with Hochheim separating from the other populations. At higher K values, further subdivisions appeared, with Langgöns and Pohlheim gradually differentiating from the Core.

Bottleneck analysis under the TPM model showed no evidence of recent bottlenecks in Core, Extirpated, Langgöns, Pohlheim, or Reference groups ($p > 0.05$), while Hochheim showed a significant signal ($p = 0.0103$), suggesting recent genetic erosion. Inbreeding coefficients (FIS) based on microsatellites were highest in the Extirpated group, followed by Core and Reference populations. SNP-based FIS values were particularly elevated in satellite populations. ROH analysis revealed short segments in the Reference and Core groups—indicative of ancient inbreeding—while some satellite individuals displayed longer homozygous stretches, pointing to recent inbreeding events.

Our results demonstrate that population size, isolation, and extinction history significantly affect genetic diversity and structure in *C. cricetus*. Satellite and extirpated populations show signs of genetic erosion and elevated inbreeding, while core populations retain comparatively higher diversity. The integration of microsatellite and genomic approaches provides a comprehensive view of the species' genetic health and supports the development of targeted conservation strategies in fragmented landscapes.



Pervasive and recurrent hybridisation mitigates inbreeding in Europe's most threatened seabirds

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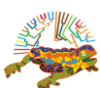
POSTER SESSION 2 - **P02.158**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Hybridisation is a double-edged sword: while it can erode distinct evolutionary lineages, it can also introduce genetic diversity and enhance the adaptive potential of dwindling populations. In the Critically Endangered Balearic shearwater (*Puffinus mauretanicus*), this dilemma is exacerbated by a limited understanding of the extent and consequences of hybridisation with the Yelkouan shearwater (*P. yelkouan*). This knowledge gap has limited the scope of effective science-based conservation strategies to avoid the Balearic shearwater's imminent extinction.

Here, we investigate shearwater hybridisation dynamics and their impact on genome-wide diversity in the Balearic shearwater. Divergence dating, demographic modelling and admixture analyses suggest that these two poorly-differentiated shearwater lineages experienced recurrent episodes of divergence and widespread hybridisation driven by climatic fluctuations during glacial cycles. Selection

scans reveal a 500 kb genomic region hosting an adaptive haplotype that potentially underpins interspecific differences in migratory behaviour, and which has been repeatedly introgressed between taxa, likely contributing to the species' adaptive potential. Moreover, we show that interspecific gene flow has also mitigated increases in homozygosity and genetic load. Through forward simulations we illustrate how hybridisation can buffer the negative effects of future population bottlenecks in the Balearic shearwater, be it via the protection of hybrid populations or via human-mediated translocation.

Overall, our findings illustrate how introgression can be crucial for maintaining genetic diversity and adaptive potential in threatened taxa, and highlight the urgency of reevaluating hybridisation dynamics in conservation actions to ensure species' survival.



Population cross-breeding improves adaptive potential in the sand lizard (*Lacerta agilis*)

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POSTER SESSION 2 - **P02.160**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

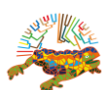
Background: The sand lizard (*Lacerta agilis*), a widely distributed species in Eurasia, is declining in most of its range due to environmental change. The Swedish sand lizard populations represents the species northernmost limit, and lizards here have adapted to the cool northern climate. Populations in the south of Sweden are relatively large and widely distributed, but further north populations become small and isolated. Environmental change has affected gene flow and inbreeding depression has been observed in populations. Approx. 25 years ago, an admixed population was established on an isolated island via cross-breeding of individuals from Asketunnan, a population suffering from severe inbreeding effects, with individuals from other Swedish populations. The offspring were released on the island of Stora Keholmen. This admixed population has since thrived on the island and any inbreeding effects of its main source population have been eliminated. In this study we investigated the genomic consequences of this human-assisted admixture.

Materials and methods: We used low-coverage whole genome sequencing of the admixed Stora Keholmen population and of its main source population Asketunnan. The Stora Keholmen individuals included in this study were sampled approx. 10 years after population establishment. For the Asketunnan population, we included samples from the time of cross-breeding and approx. 10 years later to investigate the evolutionary trajectory of the source population. We estimated genotype likelihoods jointly for all populations to obtain a set of shared sites. We then imputed missing genotypes with STITCH followed by Beagle 3. We combined this dataset with a data from additional Southern Swedish populations to infer population structure with PCAngsd and admixture with NGSadmix. We estimated genotype likelihoods with Angsd for each population. We then estimated population diversity, neutrality test statistics and population

divergence for each chromosome and for sliding windows along the genome with Angsd. We estimated inbreeding coefficients and heterozygosity for each individual with ngsF and Angsd.

Results: We found that the admixed Stora Keholmen population clustered more closely with Southern Swedish populations than with its main founder Asketunnan. Despite that, the admixture analyses suggested that they have diverged from other populations, forming a separate cluster. Interestingly, the modern Asketunnan population presented new admixture clusters compared the population at its earlier sampling time point. The population diversity analyses supported the results of the population structure analyses. Individual heterozygosity and nucleotide diversity were considerably higher in the Stora Keholmen population compared to both temporal datasets for Asketunnan, as well as other Swedish sand lizard populations. This was particularly evident for functionally important regions, such as those involved in the immune response. In line with the appearance of a new cluster in the admixture analyses, Tajima's D of the modern Asketunnan population suggested that it may be expanding following a bottleneck.

Conclusions: Our results indicate that cross-breeding of several Swedish sand lizard populations has increased genetic diversity within the admixed Stora Keholmen population. They further suggest that the Stora Keholmen has evolved in response to the conditions of their new habitat. This suggests that the human-assisted gene flow has improved the adaptive potential of the admixed population, which will aid their continued adaptation in the face of climate change. Our study is an encouraging example that genetic rescue via human-assisted admixture can be a powerful conservation strategy to ensure persistence and recovery of sand lizard populations.



Soil characteristics shape optimal migration scenarios for assisted gene flow of crops vulnerable to climate change

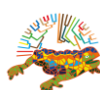
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POSTER SESSION 2 - **P02.165**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The predicted negative impacts of climate change on the adaptive potential of populations are often quantified in terms of "genetic offsets", which measure the difference between the current allelic composition of locally adapted populations and the allelic composition that would be required for adaptation to future climate conditions. Considering future climate conditions at different geographic locations has the potential to inform assisted gene flow, by minimising the genetic offset between a current source population and its hypothetical future destination. Generally assumed to approximate the (loss of) fitness in the future environment, genetic offset estimates tend to be predicated solely on present and future climate data. As such, there is a risk that identified migration scenarios fail to take into account other environmental factors that differ between

the source and destination sites, that can nonetheless influence fitness. Here, we assess the relative importance of considering soil characteristics, in addition to climate conditions, for inferring assisted gene flow scenarios in pearl millet. Relying on phenotypic data from a common garden experiment as a ground truth for reproductive success, we show that differences in soil characteristics at the site of origin can explain variation in fitness almost as well as - and augments the explanatory power of - differences in local climate. These results suggest that diverse environmental selection pressures operating outside the climate regime can influence the suitability and likely outcomes of identified migration scenarios, and determine the success of assisted gene flow as a mitigation option for the negative impacts of climate change.



Using novel selection strategies to bolster climate resilience in restoration planting stock

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*POSTER SESSION 2 - **P02.161**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM*

Plant materials used for habitat restoration and reforestation frequently respond less favorably to changing climatic conditions than invasive species that they are intended to compete with. In the context of prairie rangeland management, the presence of invasive annual grasses instead of perennial native bunchgrasses leads to landscapes that burn more frequently and intensely, are less beneficial to livestock, more susceptible to erosion, and less biodiverse. A method for supplementing genetic diversity—Assisted Gene Flow (AGF)—has shown promise in strengthening the climate resilience of plants and animals by facilitating genetic exchange that would occur slowly or fail to transpire in nature. Autogamy Selection (AS) is a selection scenario recently identified that arises from somatic mutations accumulating in the apical meristem of selfing plants and can lead to rapid adaptation. This study applies different combinations of AGF and AS treatments to

overcome the adaptational lag in a native bunchgrass ecotype that is exhibiting increased mortality as its habitat becomes increasingly arid. Through a combination of common garden experiments and analysis of whole-genome sequencing (WGS), we aim to assess the fitness impact of a variety of intergenerational crosses between ecotypes collected over a wide latitudinal range and encompassing AGF only, AS only, and AGF/AS treatments. Additionally, the project identifies endophyte species present on the grass using amplicon sequencing to account for their role in any increased drought tolerance in treated experimental groups. Results of this study will then be used to develop protocols for land managers to implement these selection treatments broadly on other herbaceous species to develop more heat- and drought-resistant planting stock for upland prairie habitat restoration.



A century's wait for hybrids: investigating the legacy of assisted gene flow in European beech forests

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MEETING ROOM 120+121, August 19, 2025, 11:00 AM - 1:00 PM

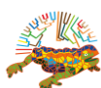
Assisted migration, the human-aided translocation of individuals within or outside their native range, is increasingly considered a necessity to help long-lived, economically important species like forest trees to adapt to rapid environmental changes. A special case of this management strategy is assisted gene flow (AGF), which aims to introduce adaptive genetic variation into a host population through hybridization with a donor species. While some countries are reluctant to use this management option, others opt for its fast implementation. The lack of consensus largely reflects our lack of knowledge on the long-term consequences of hybridization following AGF. Past introductions of non-native species may serve as natural laboratories of AGF in forest tree species. This study takes advantage of replicate introductions of beech from Greater Caucasus into European beech forests about 100 years ago in the Rhine Valley, encompassing over three generations of introgression, assuming a 30-year generation time. We studied two introduction sites, one in Switzerland and one in France, genotyping all adult trees within ~0.5 ha plots (N=216 in Switzerland, N=226 in France) and a representative number of seedlings (N=639 in Switzerland, N=423 in France) using 16 nuclear microsatellites. Bayesian clustering analysis (NewHybrids) revealed introgression rates of 13.6% and 19.6% among the offspring in the Swiss and French forests, respectively, while we detected no/uncertain introgression in adult trees.

Using a spatially explicit neighborhood model (NMpi2), we found similar dispersal ability between the two beech species and a tendency for both to preferentially mate with conspecifics, suggesting assortative mating (not significant in Switzerland). This mating preference may be partly explained by the, on

average, two-day earlier flowering of Caucasian beech, which we documented through five years of observations. Our analysis also suggested asymmetric gene flow, with hybrids more likely resulting from Caucasian beech receiving European beech pollen.

Spatial configuration, desynchronized flowering, assortative mating, slower-than-expected maturation, harvesting and selection could all contribute to the observed hybridization patterns. To disentangle the role of these factors, we developed a wrapper to perform spatially explicit simulations using Nemo-age. Our results suggest that the generation time of beech trees is well over 30 years, and the low relative abundance and clustered distribution of mature Caucasian beech trees are the primary factors limiting introgression. Further, our simulations suggest the presence of no or weak selection against hybrids in one of the stands.

Our results suggest that despite the large geographic distance separating Western Europe and the Caucasus, both adaptive and neutral genetic differentiation is small between these species. This is in agreement with recent studies that reported little difference in the growth patterns and physiological response facing water stress; yet Caucasian beech appears slightly more resistant to drought. In order to achieving widespread introgression of potentially beneficial Caucasian beech alleles, introduction of significantly more individuals distributed uniformly across the forest landscape would be necessary. Finally, the spatially explicit simulation framework developed herein provides a valuable tool for evaluating different species introduction scenarios and predicting their long-term ecological and genetic consequences.



Adaptive introgression mediates vulnerability and facilitates adaptation to climate change in Asian aspen species

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MEETING ROOM 120+121, August 19, 2025, 11:00 AM - 1:00 PM

Background: Anthropogenic global climate change is expected to adversely impact tree species biodiversity, resulting in significant biodiversity loss. A comprehensive understanding of environmental adaptation mechanisms and accurate predictions of species responses to climate change are crucial for formulating effective conservation strategies. To avoid extinction, species may shift their geographic ranges or leverage existing genetic variation for adaptation. Interspecies gene flow (introgression) is another key evolutionary process that transfers adaptive genetic variants between species, enhancing the adaptability of recipient species to increasing selective pressures. Understanding how adaptive introgression aids the adaptation of wild populations to changing environmental conditions will provide valuable insights. These insights are essential for effectively utilizing assisted gene flow (AGF) as a tool to enhance the resilience of vulnerable species and ecosystems in a rapidly changing world.

Materials and methods: To investigate the evolutionary processes shaping adaptation in the *Populus davidiana* - *P. rotundifolia* complex, key components of broadleaved forests in East Asia with a vast hybrid zone at medium altitudes, we performed high-depth whole-genome resequencing on 410 individuals from 82 populations across their distribution range. We carried out the following analyses:

(1) We examined the population structure and demographic histories of these populations. By integrating various bioclimatic datasets, we conducted genotype-environment association (GEA) analyses to identify genetic variants associated with environmental conditions.

(2) Using phylogenetic, gene flow, and selection analyses, we identified climate-induced adaptive introgression variants among divergent genetic lineages. Based on these findings, we selected a key adaptive introgressed gene, *GA2OX8* (a homologous genes of *Arabidopsis*),

along with its associated variants, for functional and phenotypic validation.

(3) We incorporated multiple evolutionary forces—including in situ adaptability, dispersal capabilities, genetic load, niche suitability, and adaptive introgression—into our predictions of how populations and lineages are likely to respond to climate change. Special emphasis was placed on the role of adaptive introgression in driving adaptation. Additionally, we evaluated the feasibility of implementing AGF to mitigate the genomic vulnerability of maladaptive populations.

(4) To validate our predictions, we are conducting common garden trials by planting trees with varying genetic structures and mixing ratios in the field.

Results: Our results demonstrate the following: (1) *P. davidiana* is divided into two lineages: Northeastern and Southwestern, with the latter (“admixed populations” hereafter) exhibits 25% introgression from *P. rotundifolia*. (2) The admixed populations demonstrate the strongest adaptive potential and lowest risk of maladaptation compared to the pure lineages of the two divergent species (Northeastern *P. davidiana* and *P. rotundifolia*). (3) The adaptive introgression from *P. rotundifolia* to the admixed populations can significantly enhance their adaptive potential, as functionally validated. (4) According to our predictions, the admixed populations may diverge from Northeastern *P. davidiana*, potentially splitting off entirely and shifting into the niche currently occupied by *P. rotundifolia*. This shift could lead to enhanced introgression and facilitate species fusion under climate change scenarios. (5) Simulated AGF could further mitigate the genomic vulnerability of *P. davidiana*.

Conclusions: This research highlights the critical role of climate-induced adaptive introgression in species' current local adaptation and their responses to future climate change, emphasizing the importance of incorporating introgression into predictive models. Our research confirms the significant value of AGF for species conservation. Additionally, we provide strong evidence that climate-mediated changes in hybridization will significantly alter the evolutionary trajectory of related species.



Restoring adaptive potential: genomic insights from a 25-year plant translocation program

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MEETING ROOM 120+121, August 19, 2025, 11:00 AM - 1:00 PM

Assisted gene flow can be an efficient tool to restore the adaptive potential of endangered populations through increasing genetic diversity, but its long-term consequences on species population viability remains controversial. *Arenaria grandiflora* L. (Caryophyllaceae) experienced a drastic decline in the Fontainebleau Forest (FOF, Ile-de-France) in the late 20th century. A preliminary study revealed that the main causes of the population's extinction were the low genetic diversity and the probable associated low fitness due to inbreeding depression. To save this population from extinction, a conservation program was launched in 1999. Local (L) plants (n=9) and non-local (NL) (Chinon, ~300km distant) individuals (n=11) were multiplied by in vitro culture, and introduced in three unoccupied sites in FOF. The same mix of 450 plants, comprising 2/3 L and 1/3 NL clones, was introduced in each site. Since then, yearly monitoring of plant's fitness was performed, coupled with genetic analyses based on microsatellite markers. Our results highlighted that the reintroduced populations presented an increased and fairly stable genetic diversity through time and displayed lower consanguinity due to higher heterozygosity level than parental ones. We found no evidence of maladaptation and, instead, a better fitness of admixed individuals compared to individuals resembling to the original clones. Finally, the proportion of NL ancestry exceeded 50% in all the extant reintroduced populations.

To expand our investigation and gain more insights into the evolutionary mechanisms shaping the observed trajectories, we first assembled a reference genome for *A. grandiflora* using long-reads and Hi-C sequencing. Next, we whole genome sequenced: i) 72 individuals sampled in the reintroduced populations (19 in 2007 and 53 in 2023); ii) some of the original clones translocated in 1999 (3 L and 4 NL); iii) 18 NL individuals from the extant Chinon

population; iv) samples from the 19th century from FOF (16 individuals) and Chinon (14 individuals) from the historical herbarium of the Muséum national d'Histoire naturelle (Paris).

Based on these new datasets, we investigate how genetic diversity, runs of homozygosity and genomic admixture patterns varied in Fontainebleau and Chinon. prior to and during the reintroduction program. Preliminary results show that extant individuals have large variability in ancestry proportions, reflecting the recent admixture history of the reintroduced populations and suggesting that the the ancestry proportions have not reached the equilibrium value expected under neutrality. Consistently, genetic diversity statistics indicated higher variance in reintroduced populations compared to the extant Chinon population. Furthermore, to quantify the relative contribution of heterosis and local adaptation in shaping the evolutionary trajectory of the reintroduced populations, we: i) estimate the mutation load and search for genomic signature of local adaptation to the Fontainebleau environment; ii) reconstruct the evolutionary trajectories of the ancestral populations in Fontainebleau and Chinon over the last centuries by means of explicit modelling approaches enhanced by the availability of historical samples. These results will shed light on the demogenetic and environmental factors that determined the population decline in Fontainebleau. Finally, we will perform forward-in-time simulations to predict the evolutionary trajectory of reintroduced populations under different scenarios, in order to inform future conservation management needs.

This study provides a unique opportunity to investigate the real-time influence of assisted gene flow on both the genetic diversity and local adaptation during a genetic rescue reintroduction program.



Will enhanced gene flow rescue populations? Multiple genetic effects interact to constrain the population benefits of increased immigration

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MEETING ROOM 120+121, August 19, 2025, 11:00 AM - 1:00 PM

Rigorously evaluating any proposal for assisted gene flow fundamentally requires understanding the full suite of genetic impacts of new individuals arriving in any focal population, and projecting the net consequences for population composition, growth rate and viability over some future timeframe. Given the increasing urgency of enacting appropriate assisted gene flow in the context of rapid climate change, such evaluations will need to be partly informed by existing long-term field studies that have already tracked genetic and population impacts of natural or assisted immigration over multiple generations, but such studies are themselves extremely rare.

We summarise and integrate key results from a comprehensive long-term study designed to quantify genetic and population dynamic impacts of immigration of diverged individuals into a focal population, utilising a song sparrow (*Melospiza melodia*) system inhabiting diverse climatic and habitat zones. We first derived new statistical quantitative genetic methods that allow partitioning of additive genetic effects of immigrants on key life-history traits from effects of pre-existing native individuals. We then utilised these methods to reveal multiple important genetic effects of immigrants, including additive genetic effects on local juvenile survival and on the mating system, alongside reductions in local inbreeding and resulting expression of inbreeding depression, then subsequent heterosis and epistatic breakdown in immigrants' descendants. We show how these multi-faceted genetic effects combined to shape the form and

magnitude of effective gene flow, revealing that net effective introgression was much greater, and much more strongly sex-biased, than inferred from the form and rate of physical immigration.

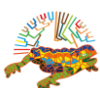
We then constructed and empirically parameterised a quantitative genetic eco-evolutionary dynamic model to project the joint outcome of adaptive evolution and population viability given different hypothetical immigration rates, such as could be generated by different degrees of assisted gene flow. Model simulations revealed how population viability emerges as the stochastic outcome of a three-way battle between extinction vortexes induced by escalating inbreeding or migrational meltdown, versus evolutionary rescue induced by sufficiently rapid adaptive evolution of local fitness. We show how these different outcomes are fundamentally shaped by the immigration rate alongside key parameters defining magnitudes of additive genetic variance, inbreeding depression and adaptive divergence alongside demographic, genetic and environmental stochasticities. Accordingly, we show how increased immigration can have counter-intuitive and unpredictable effects even within well-defined and highly biologically plausible parameter spaces. Overall, by integrating major empirical and theoretical advances, this case study sharply reveals both the potential benefits and potential challenges of assisted gene flow, and provides a comprehensive conceptual and modelling framework that can now be parameterised to infer outcomes in diverse systems.





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S27 - Genetic conflict: Evolutionary and Genomic consequences



Adaptive potential of hybridization despite initial hybrid breakdown

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POSTER SESSION 1 - **P01.318**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

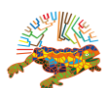
Hybridization, e.g. the interbreeding between two species or genetically distinct populations, has traditionally been considered as mostly detrimental, as it can result in genetic incompatibilities, and lead to hybrid breakdown and even extinction of species (Barton & Hewitt, 1985). However, recent studies have focused also on benefits and the possibility of hybridization enhancing adaptive potential by increasing genetic variation. This adaptive potential has been shown in many organisms, like sunflowers and fish (Mitchell et al., 2019; Brauer et al., 2023). In simulations, hybrids adapted faster to new environments than parental populations, in nearly all simulated scenarios, despite hybrid breakdown (Kulmuni et al., 2023). Still, empirical studies on the interplay between incompatibilities and adaptive potential are limited. Here, we investigated if hybrids have adaptive potential in novel environments despite such incompatibilities.

Tetranychus urticae Koch, the two-spotted spider mite, is an ideal model for tracking hybrid fitness in novel environments: they have a generation time of two weeks, they are haplodiploid, their fitness can be individually assessed, and their genome has been sequenced (Grbić, 2011). They are a generalist cosmopolitan pest, with the potential of infecting several plant species.

Because of their haplodiploidy, adaptation is thought to be quicker (Tien et al., 2015). Additionally, hybridization has been observed in wild populations.

We investigated hybrid fitness in parental and novel environments for the first three hybrid generations. Hybrids had significantly lower fitness compared to parental lines in the parental environment, indicating hybrid breakdown in the first three generations. In the novel environment, hybrid and parental fitness are similar. However, after >40 generations, we found that the hybrids outperform parental lines in a novel environment, suggesting that initial incompatibilities have been resolved and hybrids have a higher adaptive potential. We further extended this experiment to investigate if hybrids would have higher fitness compared to parents in multiple different novel environments.

In conclusion, we showed that hybridization can provide adaptive potential despite initial hybrid breakdown in *T. urticae*. With climate change, hybridization is thought to occur more, due to habitat expansion and redistribution (Chunco, 2014). Therefore, it is crucial to consider the evolutionary and ecological consequences of hybridization, especially in pest species like the two-spotted spider mite.



Causes and consequences of sex-biased reproduction in the flea beetle *Altica lythri*

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POSTER SESSION 1 - **P01.308**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Selfish genetic elements often hijack reproduction to promote their own transmission and can thereby trigger intragenomic conflict. The leaf beetle *Altica lythri* presents a striking example of such intergenomic conflict. Among four naturally occurring mitochondrial haplotypes, only HT1 and its close variant HT1* produce exclusively female offspring. The other haplotypes (HT2 and HT3), despite co-occurring in the same populations, maintain a normal sex ratio and reproduce conventionally.

This strong association between mitochondrial haplotype and sex ratio points to a unique form of sex-ratio distortion, likely driven by a combination of mitochondrial and symbiont-related mechanisms. While different *Wolbachia* strains are present in the mitochondrial haplotype lineages, our data suggest that *Wolbachia* alone may not fully explain the observed female bias. A naturally occurring variant, HT1*, shows little to no detectable *Wolbachia* infection, yet still produces 100% female offspring. This indicates that additional factors—possibly involving interactions between mitochondria, nuclear genes, and endosymbionts—may contribute to the emergence and maintenance of this sex-ratio distortion.

Paternity analyses with ddRAD approach suggest that although fertilization is

necessary for development, the paternal genome does not contribute to the next generation. This points toward a gynogenetic-like reproductive mode. Moreover, cytological analyses show that unfertilized eggs from the HT1 and HT1* retain an unreduced chromosome set, suggesting a disruption of meiosis in HT1 (HT1*) females. This phenomenon could reflect an evolved mechanism to bypass paternal inheritance altogether or a side effect of intergenomic incompatibility.

Using whole-genome SNP data from PacBio sequencing, mitochondrial and nuclear genome comparisons, we are currently uncovering the molecular players and evolutionary consequences of this system. We also explore the role of *Wolbachia*, the possibility of integrated bacterial genes, and the broader genomic signatures of conflict and co-evolution.

Understanding how mitochondria, symbionts, and nuclear genomes interact to skew reproductive outcomes will shed light on fundamental questions of sex determination, host-symbiont coevolution, and the evolutionary stability of sexual reproduction itself. The exact mechanisms still remain unclear, but our research system points toward a complex interplay of genomic influences rather than a single causative agent.



Evolution and genomics of an X-linked meiotic driver in stalk-eyed flies

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POSTER SESSION 1 - P01.305, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Meiotic drivers are a class of selfish genetic element and a classic example of intragenomic conflict. Sex-linked meiotic drivers produce massively biased sex ratios by ensuring they are inherited in more than half their carrier's offspring. However, they are often able to exist in stable frequencies through a complex evolutionary conflict between drive and suppressor alleles. Despite a century of study, the genetic basis, origins and molecular evolution of sex-linked drive systems remain poorly understood, with the exception of a few species. Here, the stalk-eyed fly, *Teleopsis dalmanni*, is an ideal system to address this knowledge gap. This species harbours an X-linked meiotic driver, stable in wild populations, that produces female-biased offspring by destroying Y bearing sperm. The driving X is characterised

by a series of inversions across its entire length, consistent with evolutionary theory linking drive and suppressor alleles. Here, we generated a large DNA resequencing dataset of drive and non-drive *T. dalmanni* males to test the evolutionary and genomic consequences of these inversions. We uncover significant sequence divergence between the drive and non-drive X with genetic patterns indicating an extended haplotype encompassing the majority of this chromosome. Importantly, we identify signatures of selection unique to each X haplotype. Understanding the genetic origins and consequences of drive will help understand its ecological impacts and interactions with sexual selection and sex chromosome evolution.



Evolutionary capacitance driven by Hsp90 during the de novo evolution of multicellularity

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POSTER SESSION 1 - **P01.303**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Hsp90 is a critical chaperone protein that maintains cellular homeostasis and stabilizes client proteins under stress. While its role as an "evolutionary capacitor" is well studied, its influence on major evolutionary transitions, such as multicellularity, remains unclear. Using the Multicellularity Long-Term Evolution Experiment (MuLTEE) with *Saccharomyces cerevisiae* (snowflake yeast), we investigated how Hsp90 contributes to macroscopic multicellular adaptation. Our study revealed that reduced Hsp90 expression promotes cell cycle delays and morphological changes, including increased cluster size and cellular elongation, facilitating the transition to macroscopic multicellularity through proteostatic mechanism. To examine this further, we engineered Hsc82-overexpressing strains (Hsc82 OE) and evolved them under settling selection for 100 daily size-based selective transfers. Despite

an initial fitness cost, all Hsc82 OE populations transitioned to macroscopic forms significantly faster than controls, with some populations exhibiting the transition within 60 transfers. Downstream analyses of evolved strains showed that Hsp90 downregulation was a convergent trait associated with adaptive changes in cellular morphology and chromosomal copy number. These findings suggest that transient Hsp90 overexpression accelerates phenotypic innovation, likely through proteostatic and epigenetic mechanisms, enabling rapid adaptation despite initial fitness trade-offs. This study highlights Hsp90's dual role as a stabilizer and driver of evolutionary innovation, providing new insights into the non-genetic mechanisms that facilitate multicellular evolution in addition to genetic adaptation.



Expression divergence in response to sex-biased selection

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*POSTER SESSION 1 - **P01.301**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM*

It remains debated whether greater degrees of sexual dimorphism would evolve if not for intersexual genetic constraints. Here we used experimental evolution to partially break the intersexual genetic constraint in *Drosophila melanogaster* to investigate the effects of a shared gene pool on the evolution of sexual dimorphism in gene expression. In six replicate populations of 1000 flies, a dominant marker (DsRed) was used to force a “Red” pool of genetically variable Chromosome 2 copies through exclusive father-to-son inheritance, while a complimentary pool of “NonRed” chromosomes was inherited primarily from mothers to daughters. After 100 generations, we demonstrated the effect of Red male-limited chromosomes in increasing male fitness through greater mating success, consistent with results from previous experimental evolution studies employing sex-limited inheritance. Differentially

expressed genes between flies with and without Red chromosomes had on average higher intersexual genetic correlations (rMF), as expected if such correlations represent a constraint to sex-specific adaptation under normal inheritance. If intralocus sexual conflict hinders the evolution of further dimorphism, we predicted that the transcriptomes of male-selected Red chromosomes should evolve to be “masculinized” relative to female-selected NonRed chromosomes. Consistent with this, splicing patterns in Red males (but not Red females) were masculinized relative to NonRed males. Contrastingly, gene expression levels were largely feminized in Red flies of both sexes compared to NonRed. We discuss alternative forms of intralocus sexual conflict that may explain these patterns.



Following the genomic traces of introgressed mitochondria in *Drosophila paulistorum*

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POSTER SESSION 1 - **P01.309**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Mitochondria are organelles that serve important functions for the survival of the cell and the organism, such as energy production, cell signalling and metabolism. To serve these functions, mitochondrial proteins encoded by the mitochondrial genome (mt proteins) must be compatible with mitochondrial proteins encoded from the nucleus (N-mt proteins). The proper interaction between those two types of mitochondrial proteins may be disrupted when species hybridize and mt proteins are introgressed into a new nuclear environment. Specifically, energy production of the cell via Oxidative Phosphorylation (OXPHOS) may be disrupted if the N-mt proteins and mt proteins comprising the OXPHOS complexes I-IV are incompatible with each other, causing great fitness costs.

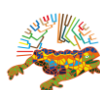
To understand the effects of introgression on mitochondrial functional integrity, we use *Drosophila paulistorum* as a study system. *D. paulistorum* belongs to the willistoni group of Neotropical *Drosophila* and comprises six semi-species with overlapping distributions throughout Central and South America. Baiao et al., 2023, showed that the mitochondria of *D. paulistorum* are polyphyletic and have two distinct mitotypes, α and β . These mitochondrial patterns are consequences of multiple introgression events that occurred in *D. paulistorum* with an unidentified species of the willistoni subgroup, which led to the replacement of the ancestral mitochondria in *D. paulistorum*. Thus, this raises the

question of whether mitonuclear incompatibilities arose in *D. paulistorum* in the past. If so, we expect compensatory mutations to have accumulated in the incompatible proteins to restore proper interactions. Another possible scenario is that the N-mt proteins in *D. paulistorum* were introgressed together with the mt proteins, meaning the N-mt are non-native to *D. paulistorum* and compatible with the mt proteins at the time of introgression.

To address this, we sequenced and assembled the genomes of 19 lines of *D. paulistorum*, covering all six semi-species, as well as five genomes from three other species of the willistoni group. We compared gene tree phylogenies of N-mt proteins of the OXPHOS complexes I-IV, against the nuclear and mitochondrial phylogenies of the willistoni group. We also calculated the dN/dS ratios for the N-mt OXPHOS proteins to evaluate selective pressures and infer possible compensatory evolution. Our analysis highlights the evolutionary dynamics of mitochondrial-nuclear coadaptation in the context of introgression and species hybridization.

References:

Baião GC, Schneider DI, Miller WJ, Klasson L. 2023. Multiple introgressions shape mitochondrial evolutionary history in *Drosophila paulistorum* and the *Drosophila willistoni* group. *Molecular Phylogenetics and Evolution* 180:107683.



Genetic drift influences levels of sex-biased expression in metazoans

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POSTER SESSION 1 - **P01.316**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Females and males differ in a broad range of physiological, morphological and even behavioural traits. Since most of the genome is shared between the sexes, these phenotypic differences are thought to be caused by differences in gene expression. Sex-biased gene expression is thought to be a consequence of different evolutionary interests between females and males, and, therefore, adaptive. Theory predicts that sex bias can arise as a response to sex-specific selection and there is empirical evidence to support said prediction. This points to the idea that sex bias could be the result of resolved sexual conflict. However, patterns that have emerged from empirical studies are inconsistent. For example, reduced sexual selection resulted in both a feminised and a masculinised transcriptome. Such contradictory evidence suggests that further empirical tests are needed to understand how variable sex bias is across the tree of life. The extent to which different evolutionary forces influence sex bias is, therefore, not fully understood. Here, we draw inspiration from other contexts of selection to investigate the relative contributions of neutral and selective forces towards the evolution of sex bias across metazoans. The effective population size – N_e – describes the rate of genetic drift in the genome of a population. Species with larger N_e experience a smaller variance in allele

frequencies across generations, leading to swifter and more effective adaptation. Alternatively, when selection is sufficiently weak compared to genetic drift, alleles behave as if they are effectively neutral. For these reasons, we can use N_e as a proxy for the efficacy of selection in a given species. We apply a similar reasoning to the evolution of sex-biased expression and hypothesise that non-adaptive sex differences will readily accumulate when N_e is low. Alternatively, if most differences between females and males are adaptive, large populations are predicted to accumulate more of these differences.

To understand the roles of selection and genetic drift in the evolution of sex bias, we analysed RNA sequencing datasets of females and males for over 40 metazoan species spanning a broad range of N_e values. Our results show a positive correlation between longevity – a proxy that is inversely correlated with N_e – and average sex bias, consistent with the hypothesis that a large proportion of sex differences in gene expression might accumulate due to genetic drift. This observation highlights the importance of considering non-adaptive processes, such as genetic drift, in shaping patterns of sex-biased gene expression across species with varying effective population sizes.



How sexes diversify: Molecular drivers underlying sexual color dimorphism in cichlids

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POSTER SESSION 1 - P01.310, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Sexual dimorphism is a widespread phenomenon where males and females of the same species vary in characteristics such as size, ornaments and color patterns. Sex-specific characteristics often only develop at the onset of sexual maturity. For example, Chindongo saulosi, an African cichlid fish has a striking color dimorphism in which both sexes of the species are yellow as juveniles and only the males change color from yellow to blue during sexual maturation. Even though such color changes are quite common among organisms, there is a considerable gap in understanding the underlying genetic and developmental mechanisms that give rise to sex-specific coloration.

Materials and methods: To investigate the genetic basis of male-specific color change in *C. saulosi*, we used QTL (Quantitative Trait Locus) mapping. We hybridized sexually dimorphic *C. saulosi* with monomorphic *C. demasoni*, in which both males and females are blue, and obtained 360 F2 hybrids. We quantified the color changing ability at sexual maturity and mapped the color change using QTL approach. To understand cellular mechanism of sex-specific color change, we performed Transmission Electron Microscopy (TEM) of skin sections as well as high-resolution light microscopy of fish scales during developmental stages. We then used single-cell RNA sequencing of adult skin of *C. saulosi* to identify transcriptional differences in monomorphic and dimorphic species.

Results: By QTL mapping color change in F2 hybrids, we identified two genomic regions associated with the color dimorphism. One locus acts as a XY sex-determination system, and consists of a tandem duplication of the *gsdf* (gonadal soma-derived factor) gene which

acts as a Y allele and strongly, but not fully determines the sexual dimorphism. A second sex-independent, autosomal locus harbors several genes involved in cell migration and differentiation. The TEM and light microscopy imaging revealed that the yellow-to-blue transition is likely driven by the emergence of one type of pigment cells: the blue, iridescent iridophores. Both males and females of the blue monomorphic species showed epidermally localized iridophores in the very early stages of the development. These iridophores were completely absent in yellow females and juvenile males of dimorphic species, but began to appear in the color changing males at sexual maturity. We also that the dimorphic species had a higher abundance of pigment cell progenitor markers in our single-cell RNA sequencing data.

Conclusions: Our findings from QTL crosses, single-cell transcriptomics and TEM images point to a heterochronic shift in the emergence of epidermal iridophores, where early developmental emergence of epidermal iridophores occurs in males and females of blue, monomorphic species. On the other hand, sexually triggered, late emergence of epidermal iridophores occurs in dimorphic males and is likely driven by high abundance pigment cell progenitors found in *C. saulosi*. This shift in timing of iridophore differentiation presents as a possible mechanism driving yellow to blue sex-specific color change in males. Taken together, we present genetic as well as mechanistic basis of sex-specific color dimorphism, and propose that a heterochronic shift in pigment cell differentiation that is influenced by a combination of a sex-linked as well as autosomal loci, underlies the sexual dimorphism in adult *C. saulosi*.



Investigating the cause of non-Mendelian inheritance of the Robertsonian 6.16 chromosome in mouse

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POSTER SESSION 1 - P01.304, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

A Robertsonian chromosome is formed by the creation of DSBs and then a fusion between the long arms of two acrocentric chromosomes.

In mice the Robertsonian (Rob) 6.16 chromosome shows transmission ratio distortion, i.e., it is under transmitted when heterozygous male mice are mated to wild type females.

We generated heterozygous Rob6.16 mice to investigate how chromosomal fusions can influence their own transmission. Two hypotheses might explain the transmission distortion: 1) accumulation of SNPs within the region of reduced recombination around the fusion site, if these SNPs occur within key genes involved in fertilisation, then this may reduce the transmission of the fused chromosomes; or 2) epigenetic silencing of genes around the fusion point, again if genes involved in fertilisation are affected this could influence the transmission of the fusion chromosome. To assess the impact of deleterious SNPs in genes surrounding the

fusion points, we carried out whole genome sequencing of homozygous Rob6.16 mice. To determine the existence of epigenetic silencing of genes near the fusion point, we compared gene expression levels in spermatids between wild type, heterozygous Rob6.16 and homozygous Rob6.16 mice using T-tests and the tool DESeq2. SNPs in seven key fertility genes (Pla2g10, Prm2, Spam1, Hyal6, Slx4, Rimb3 and Tekt5) were identified, but knockout studies would be needed to determine which SNP or combination of SNPs could be causing the effect. In heterozygotes we did not observe a decrease in reads in the region surrounding the fusion point relative to wild type or homozygous mice, nor did we observe the downregulation of many genes in heterozygotes relative to wild type or homozygous mice. This indicates that epigenetic silencing is likely not responsible for the observed transmission ratio distortion.



On the roles of transposable elements in East African cichlid evolution

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POSTER SESSION 1 - **P01.319**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

East African cichlid fishes have diversified in an explosive fashion, representing one of the most phenotypically diverse vertebrate radiations. However, the (epi)genetic basis of the phenotypic diversity of these fishes remains largely unknown. Specific transposable elements (TEs) have been associated with phenotypic variation in cichlids, but their ongoing transposition, transcriptional activity, and epigenetic silencing were not known. We tackled these aspects to understand how TEs contribute to cichlid evolution.

Using eight long-read genome assemblies, we built the first cichlid pangenome graph, uncovering extensive structural variation, mostly attributable to TEs. This suggests previously underappreciated genomic diversity arising from TE transpositional activity. We also profiled the transcriptional activity of TEs, finding dynamic expression patterns in African cichlid gonads and during early development. Orthology inference revealed strong conservation of TE silencing factors in African cichlids, and an expansion of *piwil1* genes in Lake Malawi, likely driven by PiggyBac TEs. The expanded *piwil1* copies have signatures of positive selection and retain amino acid residues essential for catalytic activity. Furthermore, the gonads of African cichlids express a Piwi-interacting RNA (piRNA) pathway that targets TEs. We

define the genomic sites of piRNA production in African cichlids and find divergence in closely related species, in line with fast evolution of piRNA-producing loci. These findings suggest dynamic co-evolution of TEs and host silencing pathways in the African cichlid radiations.

Genomic studies have shown that inter-specific hybridisation has occurred repeatedly and has been crucial in African cichlid diversification. Different cichlid species can be crossed in captivity, generating fertile hybrids. However, it is unclear how cichlid inter-specific hybrids tolerate regulatory mismatches arising from the fast evolution of the piRNA pathway and TEs. We have performed reciprocal crosses between two Lake Malawi species and observed variable embryonic lethality, associated with a range of phenotypic defects. We are currently using a multi-omics approach to determine the underlying molecular basis for these defects, as well as the basis for tolerance in surviving embryos. This includes the quantification of novel transposition events in hybrids using long-read sequencing technologies. Overall, we propose that the co-evolution of TEs and host silencing pathways, in a background of recurrent inter-specific hybridisation, have contributed significantly to cichlid evolution.



Phylogenomic Reconstruction and Taxonomic Clarification of the Ustilaginales Order

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POSTER SESSION 1 - **P01.302**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Smut fungi (order Ustilaginales, phylum Basidiomycota) are a diverse group of biotrophic plant pathogens that primarily infect grasses, including major cereal crops. Many species have significant ecological and economic impacts. Despite extensive research, the phylogenetic relationships within this order remain unresolved due to conflicting gene trees and historical classification challenges. To refine the evolutionary history of Ustilaginales, we conducted a comprehensive phylogenomic analysis incorporating 55 genomes, including newly assembled high-quality genomes generated using PacBio HiFi sequencing (Hifiasm v0.19.8) and Oxford Nanopore sequencing, covering representatives from diverse host associations.

Using a comparative genomics framework, we inferred the species tree from orthologous gene sets identified by OrthoFinder v3.1.b and constructed a maximum-likelihood phylogeny using IQ-TREE v2.3.6 with the best-fit substitution models. The phylogeny includes newly sequenced smut species such as *Stollia ewartii*, *Anthracocystis heteropogonicola*, *Triodiomyces atilis*, *Ustilago filiformis*, *Anthracoidea sempervirentis*, and *Anthracoidea ferruginea*, providing an improved taxonomic framework. Our refined phylogeny resolves key backbone relationships and clarifies previously ambiguous taxonomic placements and polyphyletic groupings, allowing for a more precise definition of genera, their

evolutionary relationships, and taxonomic boundaries.

Additionally, we analyzed the genomic architecture of mating-type (MAT) loci and characterized mating system distinctions across species, highlighting lineage-specific variations in homeodomain (HD) and pheromone/receptor (P/R) loci.

To further characterize the functional diversity of Ustilaginales, we predicted secretomes using the Predector pipeline, which integrates tools including SignalP and EffectorP to identify candidate effector proteins involved in host interactions. Carbohydrate-active enzymes (CAZymes) were classified through comparative analysis with curated databases, providing insights into enzymatic adaptations for plant cell wall degradation and pathogenicity. Our analysis aims to identify genomic signatures and gene family expansions that may underlie the adaptation of *Ustilago* species to Pooideae subfamily hosts, while other genera within Ustilaginaceae may exhibit specialization toward Panicoideae, and Anthracoidea species could show distinct genomic features linked to their association with Cyperaceae.

By producing a robust phylogenomic backbone and comprehensive genomic statistics, this study establishes a valuable reference for future research on host specialization, fungal adaptation, and genome evolution within Ustilaginales.



Red Queen dynamics in the determination of recombination hotspots

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POSTER SESSION 1 - **P01.312**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Intragenomic conflict can arise without the need of selfish genomic elements. Such is the case of the conflict exemplified by the recombination hotspot paradox. In many organisms, recombination occurs recurrently in specific regions of the genome, known as hotspots. In most vertebrate species, the location of hotspots is determined by the binding of a protein called Prdm9. Different PRDM9 alleles recognize different motifs that determine its binding sites. However, recurrent recombination in particular locations is expected to erode the corresponding binding motifs. The hotspot paradox hence arises: how can there be locations of the genome constantly undergoing recombination events when such events would lead to the destruction of the locations themselves? To resolve this paradox, researchers have suggested that hotspots exist but are short-lived, with new hotspots emerging at different locations due to the appearance of novel PRDM9 alleles. This phenomenon can be modelled adequately through Red Queen dynamics. In order to sustain PRDM9-driven hotspot recombination, PRDM9 itself would need to

mutate fast enough to enable a shift in recombination hotspot location. Aligned with this prediction, PRDM9 is one of the fastest evolving genes across vertebrates. However, the mechanism through which this high mutation rate is achieved is unknown. Modelling of PRDM9 evolution under Red Queen dynamics has revealed the need for an intrinsic high mutation to generate a high turnover rate of PRDM9 alleles. We present a simulation model specifically incorporating the zinc finger array structure in PRDM9 which determines PRDM9's binding sites. By incorporating ectopic gene conversion between the zinc fingers within the array we recover an intrinsic high mutation rate, which generates a high allelic diversity and drives the succession of high frequency PRDM9 alleles observed in natural populations. The case of PRDM9 evolution can be seen as example of intragenomic conflict in which there is no selfish genomic element per se, but rather a phenotype, in this case, PRDM9-driven recombination, which is only maintained due to PRDM9's high mutation rate.



Selfish Elements and Selective Sperm: impact of haploid selection on zebrafish fitness

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POSTER SESSION 1 - **P01.314**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Diploid organisms produce haploid gametes for sexual reproduction, resulting in a biphasic life cycle. Selection at the haploid gametic stage is not well understood, and current work assumes that selection at this stage is minimal. Previous work in our lab has shown selection on sperm longevity in zebrafish results in genomic divergence between sperm pools. In this current project, we performed a large-scale selection experiment in zebrafish *Danio rerio*, where we continued selection on either long- (LAT) or short-lived (SAT) sperm across three generations in eight replicate lines per treatment and sequenced the genomes of the founding parents (F0), the F1 and the F3 offspring. We analysed the resulting data for signs of directional and purifying selection and assess transposon (TE) and genetic divergence between LAT and SAT lines. We conducted genome-wide analysis for signatures of selection using FST and Tajima's D statistical analyses. This revealed several candidate regions with strong deviations from neutrality, with some loci exhibiting elevated FST (>0.1). These regions

of high FST also had negative Tajima's D and could be a sign of positive and HS.

Overall, F1 and F3 SAT samples showed increased TE activity across the genome and TE families, with a spike in recent activity observed in F3 SAT groups compared to LAT. A candidate list of genes and TEs has been generated through analysis of sperm pools with SAT and LAT treatments, that may be under HS pressure. The mobile genome is being analysed to see if transposable element (TE) mobilisation has had a significant role in HS and rate of genetic divergence. The long arm of chromosome 4 is showing signs of protection from TE mobilisation across all experimental groups, important as this region of the genome is thought of as the sex determination region in wild but not lab zebrafish. Our study provides unequivocal evidence for the strong impact of haploid selection through directional and purifying selection over a very short evolutionary period of just four generations.



Selfish male reproduction in stick insects

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POSTER SESSION 1 - P01.313, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The transmission of genetic information is fundamental to evolution, yet it is achieved through a striking diversity of reproductive modes. The reasons for, and evolutionary significance of, this diversity remain poorly understood. One possibility is that selfish genetic elements, which promote their own transmission, create or exacerbate genetic conflict, driving the diversification of reproductive modes. Here, we demonstrate that androgenesis—where only the male nuclear genome is transmitted to the next generation—is a selfish male strategy in the stick insect genus *Bacillus*. Specifically, individuals produced by androgenesis inherit their entire nuclear genome from their father due to the exclusion of the maternal genome during embryogenesis. Using experimental

crosses in combination with microsatellite genotyping for over 2500 individuals, we show that males differ significantly in their ability to reproduce by androgenesis. Further, using genotyping-by-sequencing on a subset of males, females, and their progeny, we identify the cytological mechanisms underlying androgenesis and propose an evolutionary scenario explaining its emergence. Our findings indicate that selfish male strategies can hijack core features of sexual reproduction to establish uniparental inheritance and highlight the central role of selfish genetic elements and genetic conflict in shaping the evolution of reproductive modes.



Sexual tension over intragenomic conflict: *Bacillus subtilis* bacteria and pB32 plasmid

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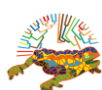
POSTER SESSION 1 - **P01.317**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Sex and recombination are key factors that impact the evolution of bacteria and their ability to acquire new genes (horizontal gene transfer). Although often overlooked in bacterial evolution, population genetic data reveal that homologous recombination occurs as often as mutation per base-pair and can vary >10x within a species. However, the evolutionary reasons for bacterial recombination remain a puzzle, raising long-standing questions in evolutionary theory about the rates of recombination and condition-dependent sex. Bacteria can undergo recombination either through the programmed cellular process of natural competence for transformation, which integrates external DNA, or via selfish mobile genetic elements, such as plasmids. Recent observational studies suggest that recombination rate variation within species is driven by intragenomic conflict between cellular competence for transformation and mobile genetic elements.

An experimental evolution approach can help us better understand this intragenomic conflict by causally testing the selective forces involved. We focus on *Bacillus subtilis*, a gram-positive model of competence for transformation, that hosts a natural plasmid, pB32. This plasmid reduces competence by ~100-fold by killing

cells in the competent state. We hypothesize that pB32 uses an abandon-ship mechanism to quickly invade naive host populations by transmitting vertically in healthy hosts but horizontally when the host experiences DNA damage. To test this, we will perform invasion experiments with wild-type and mutant plasmid variants. Evolutionary theory predicts abandon-ship recombination modifiers to evolve readily but empirical examples are lacking. If pB32 is found to employ an abandon-ship mechanism and higher rates of competence are evolutionarily advantageous for *B. subtilis*, this suggests that plasmid and host may engage in Red Queen dynamics that impact the host's propensity for horizontal gene transfer.

Mathematical models will be developed to complement our evolutionary experiments. We will extend previous theory on the evolution of recombination modifiers and mobile elements to the context of non-homologous recombination in bacteria. This project seeks to unite evolutionary theories of recombination with the bacterial way of life, helping us understand whether and why bacteria have sex. Our findings could have significant implications for understanding and controlling horizontal gene transfer.



The ‘Jump-Corruption (Gekokujō) Evolution’ hypothesis in the sex-determining gene Sry in therian mammals

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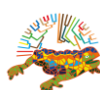
POSTER SESSION 1 - P01.306, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Sex-determining genes are highly diverse, with many arising through gene duplication or allelic variation during speciation. We previously conducted evolutionary analyses of three transcription factor-type sex-determining genes—Sry in therian mammals, dm-W in *Xenopus*, and dmy in medaka—and demonstrated that all exhibit significantly accelerated nucleotide substitution rates compared to their ancestral genes (Mawaribuchi et al., 2012). Moreover, in DM-W and DMY, we identified single amino acid substitutions under positive selection that arose independently soon after gene origination, enhancing DNA-binding ability and likely contributing to their establishment as sex-determining genes (Ogita et al., 2020). Based on these findings, we proposed the “Jump-Corruption (Gekokujō) Evolution” hypothesis: after functional enhancement under positive selection and establishment in the population, sex-determining genes may undergo nearly neutral or even slightly deleterious functional decline, potentially contributing to turnover events in sex-determination systems.

In this study, we tested this hypothesis in Sry, which likely originated from an allelic variant of Sox3 in the common ancestor of therian mammals. Focusing on the DNA-binding HMG box domain, we conducted evolutionary and functional analyses. dN/dS analysis revealed that Sry/SRY experienced more relaxed functional constraints than Sox3/SOX3, particularly along the eutherian

(placental mammal) lineage compared to metatherians (marsupials). We then generated chimeric constructs by replacing the HMG box of mouse SOX3 with those from ancestral SRY (therian and eutherian ancestors) and extant species, and assessed their DNA-binding activity in vitro. The eutherian ancestral SRY exhibited significantly higher binding affinity than either SOX3 or the therian ancestral SRY. Among twelve amino acid substitutions from the therian to eutherian lineage, two were identified as contributing to this increased affinity. Conversely, extant SRY proteins showed binding diversity: some retained or exceeded ancestral levels, while others—especially in Afrotheria—displayed marked declines. Two slightly deleterious substitutions were identified as responsible for reduced DNA binding from the eutherian to Afrotherian ancestor, with further declines observed in species such as elephants and armadillos.

These findings indicate that a specific amino acid substitution enhanced DNA-binding function from the therian to the eutherian ancestor. However, subsequent lineage diversification introduced variation, including functional reductions via slightly deleterious changes. In conclusion, our results strongly support the “Jump-Corruption (Gekokujō) Evolution” hypothesis as a compelling framework for understanding the functional evolution of Sry/SRY through alterations in DNA-binding capacity.



Biodiversity and evolution of L chromosomes using germ-line sequencing data from single wild-caught fungus gnat males

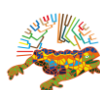
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¹Wellcome Sanger Institute, ²University College London

MEETING ROOM 120+121, August 18, 2025, 2:00 PM - 5:00 PM

Most fungus gnat species carry germ-line restricted L chromosomes (GRCs), specialised structures predominantly active in germ cells but absent in somatic cells. Despite their prevalence in this group and many cytological studies, the function, biodiversity and evolutionary origin of GRCs in fungus gnats remain unclear. Sequencing and comparison of germ-line genomes from only three Sciaridae lab colonies (*Bradysia coprophilla*, *Bradysia impatiens* and *Lycoriella ingenua*) have already revealed substantial variability in the size, number, and sequence content of these chromosomes, creating a need for more genomic data across the group. However,

establishing and maintaining inbred colonies for multiple species to obtain sufficient material for genome sequencing is often challenging, time-consuming, or even unfeasible in some cases. To overcome these limitations, we developed a protocol to capture and sequence GRCs directly from wild-caught specimens. By targeting the germ-line tissue of single Sciaridae males and employing a combination of PiMmS and ultra-low-input Hi-C methods, we are generating chromosome-level germ-line genomes for dozens of new fungus gnat species, advancing GRC research on a larger scale.



Contribution of transposable elements to misexpression of genes in *Drosophila* hybrids

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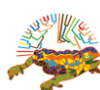
MEETING ROOM 122+123, August 21, 2025, 2:00 PM - 4:00 PM

Hybrid sterility is a fast-evolving mechanism among reproductive barriers that can reduce gene flow and accomplish the speciation of incipient species. Since this barrier is classically attributed to genetic epistatic incompatibilities, regulatory networks divergence has been proposed as a potential mechanism underlying hybrid sterility. For that matter, TE insertions have been identified as sources of regulatory divergence for their neighboring genes, through mechanisms such as altering epigenetic regulation or introducing promoter and enhancer sequences. Although the genomic stress caused by TEs aberrant activation in hybrids germline has been extensively studied, the role of TE insertions in the misexpression of their neighboring genes in hybrids remains unexplored. To address this question, we used the recently diverged sister species *Drosophila mojavensis* and *Drosophila arizonae*, that have incomplete reproductive isolation, as a model system.

Through interspecific crosses, we identified that *D. mojavensis* females produce male hybrids (MA, offspring from mother *D. mojavensis* and father *D. arizonae*) with motile sperm, as in the other cross direction, the sperm is non-motile (hybrid AM, offspring from mother *D. arizonae* and father *D. mojavensis*). However, both are sterile. Although, classically female hybrids from these species are considered fertile, we

observed that hybrid daughters from *D. mojavensis* females (MA) have decreased rates of hatching eggs. Taking advantage of genomic and transcriptomic data, we found that the X chromosome is enriched for transgressively expressed genes in both male and MA female hybrids. In addition, X chromosomes for both species have twice more TE content and genes containing insertions in their neighborhood than average autosomes. Our findings indicate that this set of genes in hybrid ovaries are enriched for TE intronic insertions. In hybrid testes, the same pattern was observed, differing on the regulation (up- or downregulation) depending on the direction of the cross (hybrids AM and MA, respectively). Additionally, we examined the presence of chimeric transcripts composed by transposable element and gene sequences, and identified chimeric reads uniquely present in hybrids. Several of these transcripts outcome from genes characterized by transgressive expression patterns. Moreover, instances of TE exonization were detected, which might further contribute to hybrid genome incompatibilities.

In summary, our results suggest that TE insertions nearby genes may drive hybrid genome incompatibilities through gene deregulation, ultimately contributing to the process of speciation.



Influence of viral infections on transposition rates in *Drosophila*

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MEETING ROOM 120+121, August 18, 2025, 2:00 PM - 5:00 PM

Transposable elements (TEs) are mobile, repetitive genetic sequences found in the genomes of all organisms, in variable proportions. Their ability to move within the genome gives them strong mutagenic potential, which can lead to deleterious effects such as double-stranded DNA breaks or the disruption of coding sequences.

Although an increasing number of effects associated with TE mobility have been identified, the full range of stimuli that induce their activation, as well as the underlying molecular mechanisms, remain to be elucidated. Some stresses result in increased TE mobilization, which can in turn amplify their mutagenic potential in the host genome. In this context, we are investigating the impact of viral infections, which constitute a biotic stress, on TE activation. In insects, TE regulation and antiviral immunity are both mediated by RNA interference pathways. Recent results from our research

group suggest that different viral infections change TE transcript levels by altering the small RNA repertoire in *Drosophila*.

TE transcription is often the first step required for transposition, however, there is no strict correlation between TE transcript amounts and transposition rates. We sought to determine whether virus-induced changes in TE transcript abundance lead to altered transposition rates. To investigate this, we quantified germline transposition rates upon viral infection in *Drosophila melanogaster* using Oxford Nanopore sequencing. In parallel, we took advantage of a genetic tool to visualize transposition in situ, and quantified the effects of viral infections on somatic transposition rates. Our data suggest that viral infections may have a direct impact on genetic diversity and genome evolution through the modulation of transposition rates.



Internal conflicts and evolutionary reversions in individuality

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MEETING ROOM 120+121, August 18, 2025, 2:00 PM - 5:00 PM

Biological beings are nested hierarchies: genes bond together into genomes, genomes within cells, cells into multicellular individuals, and individuals into eusocial groups. Different levels of biological individuality result from evolutionary transitions in individuality, in which once-independent particles bundle together into collectives, which become individualized in their own right. Cooperation is central to these transitions, but with cooperation almost always comes some room for internal conflict. In the face of these internal conflicts, can these evolutionary transitions be reverted, and if so, what happens during such an evolutionary reversion in individuality? To answer these questions, we draw on the philosophy of biology, which has produced different concepts of biological individuality. Transitions in individuality produce individuals that neatly fit with each of these concepts, but reversions may erode individuality in multiple

ways. Canonical reversions occur when a physiological arrangement formed during a transition becomes unwound, returning the once-bundled particles to their free-living state (e.g. multicellularity to unicellularity). We introduce the possibility of agential reversions, which capture a loss of individuality spurred by internal conflicts, where particles within the collective pursue their own fitness-maximizing goals in lieu of those of the collective. Such transitions need not be accompanied by a reversion in organismal form; particles don't leave the organism so much as they come to lead the organism. Agential reversions have hitherto been overlooked, despite internal conflicts being ever present. We show how these conceptual clarifications help us sketch out a research program for the study of evolutionary reversions in individuality and enable a more general approach to studying individuality and its evolution.



Multi-modal action of a mouse meiotic driver revealed by single nucleus sequencing

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MEETING ROOM 120+121, August 18, 2025, 2:00 PM - 5:00 PM

The t-haplotype is a selfish complex of genes on chromosome 17 of house mice that disables sperm which do not carry it, thus reaching 90-100% transmission ratios in heterozygous males. The phenotypic effects of the t-haplotype include sperm with altered head and tail morphologies, less progressive motility and an impaired ability to penetrate eggs. The t-haplotype is thought to work through a poison-antidote system, but the molecular players and pathways involved are not well understood. We conducted RNA sequencing at the single nucleus resolution in testes of +/t vs. +/+ males, and inferred the timing of differential expression during spermatogenesis in order to find candidate poisons and antidotes. We identified novel biological pathways affected by differential expression in the +/t germline, which involve key components of sperm motility regulation, such as dyneins, protein

phosphatase 1 and the mitochondrial cytochrome complex. Further, to investigate the possibility of differential protein phosphorylation in +/t vs. +/+ testes, we conducted the first proteomic and phospho-proteomic comparison of these genotypes. Finally, we report that dyneins, the crucial motor proteins of the flagellum, have undergone a large expression increase both on the normal chromosome 17 and on the t-haplotype in recent evolutionary times, which may be the first evidence of an arms race between these homologous chromosomes induced by meiotic drive. In line with the complex phenotypic effects and genomic structure of the t-haplotype, the results of this study suggest that several genes act together to compromise multiple aspects of sperm function and to increase the transmission rate of the t-haplotype.



Routes to Sciarid strangeness: modeling chromosome elimination and a selfish linkage group

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¹University Of Edinburgh

MEETING ROOM 120+121, August 18, 2025, 2:00 PM - 5:00 PM

Many species have separate sexes, but the genetic and physiological mechanisms determining sex are highly diverse. Sciarid flies (a.k.a. fungus gnats) are particularly fascinating in this regard because their systems of sex determination and inheritance combine various unusual traits: males eliminate their father's genome from the germline, meaning that their sperm contain only their mother's genetic material. During spermatogenesis, the X-chromosome is duplicated, so sperm end up with one set of autosomes and two X chromosomes. Since eggs also contain an X chromosome, this would result in zygotes with three X chromosomes—which are inviable as embryos. To prevent this, mothers eliminate either one or two of the paternal X chromosomes from embryos resulting in female or male development respectively. In some species, females specialize in producing single-sex broods: two genetically distinct types of females coexist with one producing only daughters and the other producing only sons. Finally, Sciarid flies also carry germline restricted chromosomes which are inherited in sperm and eggs but eliminated from somatic cells. This cooccurrence of many unusual traits

makes it difficult to decipher what evolutionary drivers caused this system to evolve, as there are no clear intermediate states. Hence, a theoretical approach to this question can identify possible evolutionary paths toward this system that can then be tested through molecular means. I suggest an evolutionary path consisting of three consecutive evolutionary transitions that could have led from a standard XX/X0 sex determination system to the complex Sciarid system. In each proposed transition intragenomic conflict causes one stable sex determination system to evolve into another, starting with paternal genome elimination, continuing with X chromosome elimination, and finally the invasion of a selfish linkage group that controls X-duplication in sperm and X-elimination by mothers. These hypotheses have implications beyond Sciarid flies because many animals share some of the features of this unusual system. For example, aphids also create male offspring through X elimination, paternal genome elimination is common in arthropods, the proposed selfish linkage group mirrors cytoplasmic incompatibility systems created by endosymbiotic bacteria.



The innovative germline-restricted chromosome of passerine birds

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MEETING ROOM 120+121, August 18, 2025, 2:00 PM - 5:00 PM

Programmed DNA elimination has been documented in songbirds (oscines) through the presence of Germline-Restricted Chromosomes (GRCs). Research on bird GRCs has focused largely on species closely related to the zebra finch, a principal model for passerine genomics. In contrast, the occurrence and properties of GRCs in other major passerine groups remain poorly characterized, with suboscines completely unexplored by cytogenetic or molecular approaches. In this study, we present the most extensive comparative investigation of GRCs to date, spanning around 50 million years of passerine diversification. Using three high-quality germline reference genomes and 25 additional germline genome drafts from all major passerine clades, we demonstrate that GRCs are found in approximately two-thirds of all bird species. Our analyses reveal that GRCs exhibit rapid and independent evolutionary trajectories distinct from the conserved synteny of A chromosomes, yet they retain a subset of

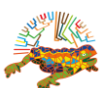
genes under purifying selection. While many genes have undergone turnover, some of them—like *cpeb1* and *pim1*—show deep conservation across lineages, indicating an early and shared origin. In contrast, genes like *mfsd2b* and *bmp15* appear to have been independently incorporated into the GRC multiple times, with *bmp15* showing up to six acquisition events, suggesting lineage-specific adaptive pressures. Notably, we identify the *zglp1* in the GRC of an ancestral lineage. This gene was originally duplicated from chromosome 30 and subsequently lost from its ancestral locus, suggesting that the GRC can carry essential genes. These results position the GRC as a dynamic and potentially selfish genomic element, shaped by and perhaps driving intragenomic conflict. Its widespread occurrence and role in germline function underscore its potential as a model system for studying the evolutionary and genomic consequences of genetic conflict across deep evolutionary time.





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S28 - Genome Architecture and Their Role in Evolution



A high-quality reference genome for the Ural Owl (*Strix uralensis*) enables investigations of cell cultures as a genomic resource for endangered species.

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¹Lib - Leibniz Institut Für Die Analyse Des Biodiversitätswandels

POSTER SESSION 2 - P02.172, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

While reference genomes are becoming available for many bird species, there are currently only a handful for the family Strigidae, which counts 310 described owl species. In this study, we generated a high-quality, chromosome-level, reference genome for the Ural Owl (*Strix uralensis*) to support conservation efforts and genomic studies within this family. Using this genome, we analysed candidate genes linked to nocturnal hunting adaptations and feather coloration and gene families that have expanded or contracted within Strigidae. We

were able to detect a chromosome fusion characteristic of the *Strix* family and annotated significant repeat expansions consistent with previously published bird and owl genomes. Additionally, we explored the feasibility of using cell cultures as a DNA source for sequencing for species with limited biological samples, a challenge often encountered in conservation genomics. This study offers valuable resources for research on Strigidae and other avian species and help guide future work on the use of cell cultures for DNA sequencing.



A novel genomic structure organising the centromeres of the haplochromine cichlid radiation

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POSTER SESSION 2 - **P02.180**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Cichlids are a family (Cichlidae) of teleost fishes distributed widely in the old and new worlds, with a particularly high diversity in the East African great lakes and surrounding region. The haplochromine cichlids (tribe Haplochromini) of Lakes Victoria and Malawi have long been considered amongst the most striking examples of recent adaptive radiations, with more than 800 species endemic to Lake Malawi that display extensive phenotypic diversity despite diverging within the last million years (sequence divergence 0.1%–0.25% between species pairs). This makes them an excellent model to study the drivers of speciation. One of the potential drivers of genetic diversity in this short timeframe is the high activity level of a wide variety of transposable element families, which lead to a very high level of structural variation (Quah FX et al., Lake Malawi cichlid pangenome graph reveals extensive structural variation driven by transposable elements. *Genome Res.* 2025).

Methods: We have recently assembled nine new high quality cichlid genome sequences using long read (Oxford Nanopore) technology, yielding many complete telomere-to-telomere (T2T) chromosomal sequences. These allowed us to annotate and compare in detail centromeric and other repetitive sequences.

Results: The cichlid centromeric sequences are readily identified as being dominated by a 237-bp satellite repeat. Strikingly, most of the haplochromine centromeres, but not those from outgroup species, have a novel structure in which the repeat units are not all oriented in tandem in the same direction. Instead, there are an inverted pair of unique sequences, separated around 150 kb apart (83 - 305 kb), from which the tandem repeats diverge, leading to four separate tandem repeat domains. These unique sequences, which we

call centroids, occur twice only per chromosome, in the centromeres, and are more similar between the pair within a chromosome than between chromosomes, suggesting that they might gene convert within a chromosome. The inward-pointing satellite arrays are more similar to each other than to the outward-pointing arrays, and the demethylated centromere dip region (CDR) is always found between the centroids. The centroid sequences themselves are ~13kb long and appear to be derived from a Tigger-like DNA transposon, although they are incomplete with respect to standard transposition requirements, so almost certainly no longer capable of autonomous mobility. They each contain a few copies of the satellite repeat that contain apparent CENP-B binding boxes. Although this structure is seen in most chromosomes, some chromosomes have lost one or both centroids, different chromosomes in different species. Centromeres lacking both centroids have much longer tandem satellite repeats, all oriented in the same direction.

Conclusion: We suggest a model where the centroids arose from a Tigger transposon to seed neocentromeres in the ancestors of the haplochromine cichlids, originally determined by CENP-B binding within the centroids themselves. Subsequently they seeded satellite expansions by meiotic drive that ultimately are sufficient to support epigenetic maintenance of centromeric function. Intriguingly, the deeply conserved CENP-B DNA binding protein itself is thought to have derived ancestrally from a DNA transposase, potentially from the Tigger element. This model of rapidly evolving centromeric sequence could provide the basis for a mechanism to support sympatric speciation based on meiotic drive and chromosome missegregation.



Bringing light into the enigmatic genome architecture of B chromosomes: germ line dynamics in Neotropical deer

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POSTER SESSION 2 - P02.204, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Understanding how genomic variation influences meiotic processes is key to decipher speciation processes. In this context, the study of heterokaryotypes provides valuable insights into how chromosomal variation, including polymorphisms and non-standard elements, is transmitted and maintained during the formation of the germ line. Among these are B chromosomes, dispensable karyotypic elements that do not follow Mendelian rules of inheritance. They can vary in number both between individuals and among cells or tissues within the same individual. Only about 1.94% of mammalian species possess them. This type of chromosomal variability is particularly well documented in mammalian species belonging to Neotropical deer (Artiodactyla: Cervidae), which exhibit considerable variation in chromosome number and genome structure, often displaying polymorphisms such as chromosomal fusions and the presence of B chromosomes. Yet, studies on the meiotic behaviour and recombination of B chromosomes in mammals are still scarce. Here, we investigate the B chromosome

meiotic dynamics in Neotropical deer through a cytological analysis focused on meiotic prophase I. This included the analysis of chromosome synapsis and recombination in a male individual of the brown brocket deer (*Subulo gouazoubira*, $2n=70+0-3$ Bs) and individuals of the red brocket deer complex (*Mazama americana* and *Mazama rufa*, $2n=42-53+2-6$ Bs). To detect recombination at A and B chromosomes, we employed immunolocalization of MLH1. This approach allowed us to observe the dynamics of B chromosome segregation, with regular MLH1 foci detected on these chromosomes, further supporting the occurrence of recombination in mammalian B chromosomes. Through this investigation, we have provided new insights into the meiotic behavior of B chromosomes, including their pairing, recombination, and segregation dynamics. We anticipate that our results will provide a new interpretative hypothesis on how meiosis can influence the inheritance and persistence of the enigmatic genome architecture of B chromosomes.



Characterizing sex-chromosomes and the sex-linked region in *Wikstroemia sandwicensis* from Hawai'i island

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POSTER SESSION 2 - **P02.189**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Wikstroemia is a genus of flowering plants in the family Thymelaeaceae, with a wide distribution throughout Asia, Oceania, and Polynesia. The vast majority of species in this group are hermaphroditic, with the notable exception of those species that have evolved in the Hawaiian Islands, where nine out of twelve endemic species have physically separate sexes (dioecy). The Hawaiian Islands were colonized once by a hermaphroditic ancestor, from which dioecy evolved in several different lineages independently. Previous greenhouse crossing studies of Hawaiian species have established that sex expression in the dioecious species is governed by genetic sex determination, and these results strongly indicate XY systems in all cases. However, the identity of these sex chromosomes and the genes involved in sex determination are unknown. In this project, I investigate the genomic basis of sex determination in *W. sandwicensis*, a dioecious species from Hawai'i island.

For this study, I first generated high-quality reference genomes for one male and one female, using whole-genome sequencing (PacBio HiFi). A combination of methods using the genome assemblies and Illumina short-sequence data for 12 females and 22 males were performed to identify the sex-linked region of the genome. Bioinformatic methods used were analysis of coverage differences between males and females,

male-female divergence estimates (FST), reference-based SNP density, k-mer analysis to identify male-exclusive regions and GWAS. The size of the sex-linked region and its degree of degeneration were unknown at the start of this project, which made it necessary to perform several analyses providing multiple lines of evidence for sex-linked inheritance.

The sex-linked region in this species is small and does not appear to be associated with any major chromosomal rearrangements. The results of these analyses allow us to characterize the sex chromosome pair in *W. sandwicensis*, as well as locate the sex-linked region in the specific chromosomes. In combination with annotation of the protein coding genes, this enables us to suggest candidate sex-determining genes for the species, which opens the possibility to do functional analyses of these genes. Overall, this study provides the first genomic analyses of the sex-linked region in this species and paves the way for a deeper understanding of sex determination in *W. sandwicensis*. It also represents the first step in a comparative study of dioecy in the group. Further analyses in other species of the group will help us infer the evolutionary history of dioecy in the Hawaiian *Wikstroemia* and increase our understanding of the transition from hermaphroditism to dioecy in plants.



Chromosomal rearrangements and their role in rapid sex chromosome turnovers in African cichlid fishes

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POSTER SESSION 2 - **P02.183**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Sex chromosome turnover can be associated with major changes in genomic structure, such as inversions, translocations and chromosomal fusions, however whether these are a cause or consequence of turnover remains unclear. The Lake Tanganyika radiation of cichlid fishes shows a remarkably high rate of sex chromosome turnover between closely related species, providing a prime resource to investigate this dynamic. Within one tribe of this radiation, Cyprichromini, phylogenetic reconstruction reveals a sex chromosome turnover from an XY system to a ZW system involving the same chromosome pair, and a subsequent turnover involving fusion. We generated haplotype-resolved whole genome assemblies of three species of this tribe, and combined this with cytogenetic investigations, to establish the precise nature of these transitions, and understand the impact of genomic rearrangements. Both *Cyprichromis microlepidotus* and *C. coloratus* share LG05 as their sex chromosome, however acting alternatively as an XY and ZW pair, respectively in either species. We compared gene content,

synteny and phylogenetic relatedness of these sex chromosomes and found a large translocation between the X and Y chromosome in *C. microlepidotus*. Meanwhile in *C. coloratus* we found a large inversion between Z and W pair, which share one of the same breakpoints as the XY translocation. Based on phylogenetic reconstruction and synteny analysis, we conclude that the W chromosome evolved from the X chromosome of these species, and the inversion then formed the Z chromosome. *C. leptosoma* also shares the LG05 ZW system, but fused to another chromosome pair, and we find that this was also associated with further chromosomal fusions in this species. Taken together, this dataset allows us to pinpoint major changes in genome architecture associated with heterogamety transition and neo-sex chromosome formation that occur early in sex chromosome evolution. Given the disproportionately large role sex chromosomes play in speciation, this exemplifies how rapid sex chromosome evolution in cichlids may have contributed to their equally rapid diversification.



Chromosomal structure and parallel evolution in the largest known avian sex chromosomes

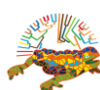
Jacobsen Ellerstrand S¹, Fu Y¹, Proux-Wéra E², Alström P³, Hansson B¹

¹Lund University, ²Stockholm University, ³Uppsala University

POSTER SESSION 2 - **P02.210**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Avian genomes are relatively well conserved across evolutionary time, with stable sex chromosomes and few genomic rearrangements. Yet, larks (Alaudidae) have enlarged neo-sex chromosomes formed by three autosomal translocations to the ancestral avian sex chromosomes. Here, we present a chromosome-level assembly of the Skylark (*Alauda arvensis*) and describe its neo-Z chromosome in the context of avian genome evolution. Further, we use whole-genome data of female-male pairs of 40 additional species in the lark phylogeny to describe variation in recombination cessation across their neo-sex chromosomes. We find a surprising number of repeated and independent events of

recombination cessation across the lark family. We reveal additional autosomes with patterns of sex-linkage in the Red lark (*Calendulauda burra*), thereby resulting in a new candidate for the largest avian neo-sex chromosome formed by (parts of) five chromosomes. This species adds to an exceptional case of repeated, independent translocation of chicken chromosome 4 to avian sex-chromosomes. Our results suggest that genome evolution in some avian lineages can be dynamic, and that some chromosomal regions host genes which are predisposed to repeatedly partake in the formation of sex chromosomes over evolutionary time.



Chromosome-Level Genome Assemblies Reveal Sex Chromosome Dynamics in Rapidly Evolving Spined Loaches (Cobitis)

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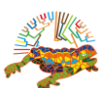
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POSTER SESSION 2 - **P02.212**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Reproductive strategies in vertebrates span from canonical sexual reproduction to diverse forms of clonal inheritance, particularly in hybrid lineages. In many of these systems, recombination between homologous chromosomes is suppressed, resulting in non-Mendelian inheritance patterns and unusual genomic consequences. These can lead to all-female parthenogenetic lineages, which provide powerful models for studying the genetic basis of sex determination, reproductive isolation, and the emergence of asexuality. Spined loaches of the genus *Cobitis* are an excellent model for exploring the connections between speciation, hybridisation, sexual reproduction, and asexuality, with multiple cases of interspecific hybridisation documented across Eurasian hybrid zones involving species that diverged 1-15 million years ago. Despite this, no sex chromosomes have been characterised in the group to date, representing an important gap in understanding the mechanisms underlying rapid speciation and hybrid sterility. In this study, we generated high-quality, chromosome-level genome assemblies for three parental *Cobitis* species (*C. elongatoides*, *C. taenia*, and *C. tanaitica*), which give rise to diverse asexual hybrid lineages. Male genomes were sequenced using Oxford Nanopore and polished with Illumina reads, while Hi-C data enabled chromosomal scaffolding. Pooled whole-genome sequencing from both sexes across all species was used to detect sex-specific regions through coverage and SNP pattern analysis.

In *C. elongatoides*, our analysis revealed a clear sex-linked signal across the entire chromosome 1 (Ch01a) scaffold, suggesting a differentiated XY sex chromosome system. In *C. taenia*, a putative Y-linked region was identified on chromosome 5 (Ch05) based on male-specific genomic coverage, pointing to a young and minimally differentiated sex chromosome system. In *C. tanaitica*, sex-specific SNP analysis revealed a candidate region on chromosome 6 (Ch06) showing moderate

differentiation between the sexes. This suggests the presence of a partially differentiated, intermediate-stage sex chromosome system, although a clear coverage signal or PCR validation is still needed. Sex-specific SNPs and coverage differences were used to design PCR primers targeting putative X- and Y-linked loci in *C. elongatoides* and *C. taenia*, three of which reliably distinguished between male and female genotypes. Additionally, we mapped known actinopterygian sex-determining genes and identified several strong candidates (such as *paics* – the master sex-determining gene in blue tilapia) located in differentiated regions of the putative sex chromosome in *C. elongatoides*. These results support the presence of distinct, nonhomologous sex chromosome systems in three closely related *Cobitis* species and highlight the dynamic nature of sex determination mechanisms in teleost fishes. Moreover, since these species with nonhomologous sex chromosome systems successfully hybridise in nature, we used validated PCR markers to investigate how the merging of divergent sex-determining regions affects sex differentiation in diploid and triploid *C. elongatoides* × *C. taenia* hybrids. While most hybrid males carried the expected Y chromosome signal and females did not, we observed notable exceptions. These included wild F1 individuals lacking any signal from either parental species and some females testing positive for Y-linked markers. These unexpected results reflect the fluidity of sex-determining systems in hybrid genomes and suggest that non-Mendelian inheritance or structural variation may disrupt standard patterns in sex-linked regions, warranting further investigation. Prior to this study, evidence for genetic sex determination (GSD) in the Cobitidae family was limited. Our findings indicate rapid sex chromosome turnover and a potential role for GSD in hybrid sterility and the evolution of asexuality.



Comparative genomics illuminates karyotype and sex chromosome evolution of sharks and rays

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POSTER SESSION 2 - **P02.199**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Chondrichthyes is an important lineage to reconstruct the evolutionary history of vertebrates. We analyzed genome synteny for nine chondrichthyan chromosome-level genomes. Our comparative analysis reveals a slow evolutionary rate of chromosomal changes, with infrequent but independent fusions observed in sharks, skates, rays and chimaeras. The chondrichthyan common ancestor had a proto-vertebrate-like karyotype, including the presence of 18 microchromosome pairs. The X chromosome is conserved across the subclass Elasmobranchii, with fusion events involving autosomes and proto-sex chromosomes occurring in only a few ray species, giving rise to an X1X2Y sex determination system. This indicates that

the oldest vertebrate sex chromosomes can be traced back to approximately 300 million years ago. We characterized the Y chromosomes of two sharks that are highly differentiated from the X, except for a small young evolutionary stratum and a small pseudoautosomal region. Compared to sharks, rays with autosome–sex chromosome fusions possess a longer pseudoautosomal region and exhibit more complex evolutionary strata. We found that shark sex chromosomes lack global dosage compensation, but dosage-sensitive genes are locally compensated. This study advances our understanding of sex chromosome evolution in Elasmobranchii and vertebrate chromosome evolution.



Divergent sex chromosome systems in Australian marsupials – implications for evolution

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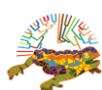
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POSTER SESSION 2 - **P02.203**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

During meiotic prophase I, homologous chromosomes pair, synapse and recombine in a tightly regulated process, which ensures the generation of genetically variable haploid gametes. This process has canonical features that are highly conserved across mammals, although there are notable differences between taxa. The mechanisms underlying meiotic cell division have been thoroughly studied in many model species. However, our understanding of the dynamics of meiotic prophase I in non-traditional model vertebrates is still in its infancy, especially when viewed from a phylogenetic perspective. Here, we compare the similarities and differences in the regulation of meiosis in three Australian marsupial species with different sex chromosome systems: the tammar wallaby (*Macropus eugenii*: family Macropodidae) and the fat-tailed dunnart (*Sminthopsis crassicaudata*: family Dasyuridae), both with an XY system,

and the greater bilby (*Macrotis lagotis*: family Thylacomyidae) with a neo-XY system resulting from a fusion between an autosome and ancestral sex chromosomes. Here, we performed a cytological analysis of the meiotic prophase I, including the study of chromosome synapsis, double strand break (DSB) formation and meiotic sex chromosome inactivation (MSCI). Our results show that sex chromosomes associate forming the so-called dense plate following different strategies in marsupial species, which correlates with differential sex chromosomes architecture and transcriptional patterns. Remarkably, we detected that the regulation of sex chromosome silencing through MSCI varies considerably among marsupial species with different sex chromosome systems, which has important implications for sex chromosome evolution.



Evolution of Gene Regulatory Networks under Negative Frequency-Dependent Selection

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POSTER SESSION 2 - P02.205, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Gene regulatory networks (GRNs) describe the mutual effects of gene expression among different genes, enabling organisms to adapt to their environment by differentially responding to external inputs. Modelling studies suggest that these networks evolve in response to various selection pressures. However, how GRNs adapt to negative frequency-dependent selection (NFDS), where rare phenotypes are favoured, is yet to be studied. Specifically, the evolutionary constraints and mechanisms that shape GRN architectures to facilitate the maintenance of polymorphism remain poorly understood. Using computational and mathematical models, we contrast the evolution of GRNs under stabilising selection and NFDS. Preliminary results suggest that while gene expression directly affects phenotypic fitness and is therefore subject to natural selection, network topology undergoes indirect selection due to its

influence on properties such as evolvability and robustness.

This project employs individual-based simulations, where each individual has a unique GRN subject to mutations, describing the mechanisms of gene expression regulation. Our approach aims to determine whether key topological features of real-world GRNs (eg. modularity and sparseness) can emerge from historical selection on gene expression patterns. We also analyse how this architecture is inherited through both sexual and asexual reproduction. Beyond this, we explore whether NFDS promotes greater evolvability and robustness compared to stabilising selection. Ultimately, this work seeks to elucidate how the architecture of GRNs responds to distinct selection pressures and its broader implications for evolutionary ecology.



From Genome to Chromosomes: Satellite DNA Mapping and Comparative Cytogenomics in *Engystomops pustulosus* (Anura, Leptodactylidae)

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POSTER SESSION 2 - **P02.206**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Advancements in sequencing technologies have enabled the generation of vast amounts of genome data. In the case of anurans, the generation of high-quality genome assemblies is still in its early stages, as is the integration between genomic and cytogenetic data. *Engystomops pustulosus*, a species of the Leptodactylidae family, is one of the few species of anurans with a chromosome-level genome assembly available, but little is known about its karyotype or its fraction of repetitive sequences. In this study, we integrate genomic and chromosomal data to resolve discrepancies between public genomic resources and cytogenetic data available for *E. pustulosus* by analyzing the distribution of satellite DNAs (satDNAs) in the genome assembly and metaphase chromosomes. We used the RepeatExplorer pipeline to analyze the whole genome sequencing reads of this species and identified 58 satDNAs. Four of these satDNAs, the PcP190 satDNA, and nucleolar ribosomal DNA sequences (rDNA) were mapped to the genome assembly using Chromosomal in silico Mapping (CHRISMAPP) and to chromosome preparations using fluorescent in situ hybridization (FISH). EpuSat2 was clustered centromeric to all chromosomes. EpuSat3 coincided to nucleolar organizer regions (NORs) also revealed by the Ag-NOR method. EpuSat13 was found in pericentromeric regions of four chromosomes. PcP190 was present in the pericentromeric region of 1p and in both arms of the NOR-bearing chromosome (chr.) 9. At last, EpuSat16 was clustered at the terminal regions of most chromosomes, except for chr. 11. Comparative analysis between the in situ and in silico mappings revealed high concordance in chromosomal localization of these sequences, except for the NOR, since the rDNA loci and associated satDNA (EpuSat3-124) are underrepresented in the genome assembly.

While FISH detected EpuSat3 and nucleolar rDNA on 8q, 9q, and 11q, the assembled genome did not show any 28S rDNA unit, chr. 8 showed few 5.8S rDNA and 18S rDNA units, chr. 9 showed a unit of 18S rDNA, and chr. 11 had no rDNA annotated. Only EpuSat3 was found on the assembly of chrs. 8, 9, and 11. This discrepancy likely reflects limitations of genome assembly derived from repeat-collapsing algorithms, which systematically underestimates the abundance of repetitive elements. In contrast, EpuSat16 appeared clustered at all terminal/subterminal regions in the genome assembly, whereas FISH failed to detect three of these sites. Based on the distribution of centromeric and pericentromeric satDNAs, we observed that the genome assembly incorrectly orients chrs. 1, 2, 7, and 8, starting with long-arm sequences rather than short-arm sequences. Notably, 18S rDNA was mapped to the beginning of chr. 8 assembly, while FISH and the Ag-NOR method detected NOR on 8q, corroborating the inversion in the assembly. In contrast, for chr. 9 assembly, although the mapping of the centromeric EpuSat2 could also suggest an incorrect orientation, nucleolar rDNA and EpuSat3 mapped downstream EpuSat2, while PcP190 mapped upstream the centromeric marker, which is consistent with the FISH results for 9q and 9p, respectively. These findings support the hypothesis that the NORs are underrepresented in the genome assembly, likely leading to an underestimation of the length of 9q. At last, we highlight that the markers on chr. 9 were helpful in distinguishing it from chr. 8 in the cytogenetic analysis, as both chromosomes are similar in size and morphology. Therefore, the combined genomic and cytogenetic analyses enabled accurate identification of the *E. pustulosus* chromosomes, which may be highly useful for further karyotype comparisons.



Genome assembly and annotation of the parasitoid jewel wasp *Nasonia oneida*, with insights into the genomic evolution of *Nasonia* species

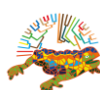
Rana A¹

¹liser Mohali, ²liser Mohali

POSTER SESSION 2 - **P02.211**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The jewel wasp, *Nasonia* (Hymenoptera: Pteromalidae) is a well-established model system for evolutionary genetics and host-microbial interactions. Of the four species, *N. oneida* and *N. giraulti*, are sister species, estimated to have diverged 0.4 MYA, and can interbreed without any detectable intrinsic post-zygotic isolation, and can serve as a powerful tool for the genetic mapping of different phenotypes. Genome sequences are available for three of the species except *N. oneida*. In this study, we detail the annotated genome of *N. oneida* by using 10× Genomics linked-read (400X coverage), Illumina short-reads (120X coverage), and transcriptome data (30X coverage) generated from both sexes across various life stages. The assembled genome is 267 Mb in size spread across 4,679 scaffolds, with an N50-scaffold size of 1 Mb. The completeness of this assembly is 98.4% when compared to arthropod-conserved BUSCOs (Benchmarking Universal Single-Copy

Orthologs). Annotation of this assembly identified 32.29% (86.46 Mb) of repetitive sequences and 14,222 protein-coding genes. Comparative genomic analyses with other hymenopterans showed significant expansions in gene families related to chemosensory and detoxification, which is consistent with *Nasonia* evolution. Genes undergoing positive selection that were identified in *N. oneida* and *N. oneida/N. giraulti* lineages, indicate enrichment of the Hippo signaling pathway and circadian rhythm-associated genes. As observed in other *Nasonia* species, genes involved in nuclear-mitochondrial interactions also show signals of positive selection in *N. oneida/N. giraulti* lineage. This study provides the first de novo assembly of *N. oneida* and is a significant addition to the growing repertoire of molecular tools for comparative genomics and functional studies in understanding the evolution of closely related species.



Genome defence and expansion – Repeat-Induced Point mutations shaping genome-wide TE activity in a ubiquitous ectomycorrhizal fungus

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POSTER SESSION 2 - P02.173, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Cenococcum geophilum is one of the most widespread ectomycorrhizal fungi in temperate forests, yet the molecular mechanisms underpinning its adaptation and symbiotic interactions remain poorly resolved in view of its large, repeat-rich genome. We assembled a chromosome-level, telomere-to-telomere reference genome of the *C. geophilum* reference strain 1.58 using PacBio HiFi and Hi-C technologies, resulting in a 178.54 Mbp genome with seven contiguous chromosomes. We identified 14,145 genes and found that over 70% of the genome consists of transposable elements (TEs). LTR retrotransposons (47.0%), LINEs (6.7%), and DNA transposons (5.9%) dominated, with three main TE families (Ty3 [61.88 Mbp], Ty1 [20.01 Mbp], and Tad1 [11.25 Mbp]) contributing over 60% of the genome size, indicating recent transposition bursts. We then searched for TE insertions in 15 *C. geophilum* strains and detected considerable variation between chromosomes and between TE families across strains, supporting lineage-specific

TE dynamics. Looking at genome defence mechanisms against TE proliferation, our analysis of repeat-induced point mutation (RIP) revealed that over 81% of *C. geophilum* genome was affected by RIP, yet several TEs remain intact and potentially active. Expression profiling across fungal tissues revealed that 55 TEs were differentially expressed between ectomycorrhizal and free-living mycelium tissues. This number increased to 75 significantly differentially expressed TEs when comparing sclerotia (stress-adapted structures) with free-living mycelium tissues, highlighting the dynamic regulation of TEs in response to symbiotic and stress-related conditions. Our outcomes demonstrate that the *C. geophilum* genome expansion was driven by a few TE families over a recent period and that expansion has been lineage-specific in respect of TE abundance and diversification. We also highlight the potential influence of TEs on key symbiotic functions and traits associated with desiccation resistance.



Genome evolution in a karyotypically diverse primate group and the role of chromosomal fissions as reproductive barriers

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POSTER SESSION 2 - P02.186, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The fact that divergent species often have different chromosome numbers in combination with observations of sterile heterokaryotypic hybrids gave rise to the classical model of chromosomal speciation. Moreover, positive correlations between the rates of chromosomal evolution and speciation have been established in several groups, reinforcing the idea that karyotypic changes may drive species divergence. Nevertheless, the mechanisms that make some lineages more prone than others to chromosomal fissions and fusions – and the causal role of such events in speciation – remain poorly understood. We study chromosomal evolution and its role in speciation in guenons (tribe Cercopithecini), a speciose and karyotypically diverse clade of African primates. Over the last ca. 12 million years, guenons diversified into ~35 extant species with diploid chromosome numbers ranging from 48 to 72. We generated de novo genome assemblies for eight guenon species and incorporated published genomes into a data set consisting of chromosome-level assemblies from eleven species with different karyotypes. A synteny analysis revealed at least 27 independent chromosomal fissions and three fusions throughout the guenon evolution. Several fissions show patterns among extant species that are discordant with the guenon phylogeny. Since recurring fissions are presumably rare, these discordances more likely result from incomplete lineage sorting or introgressive hybridization – two processes that are unlikely under a strong underdominance effect. Our results therefore suggest that these fissions were well tolerated

in the ancestral populations, questioning their role as reproductive barriers. However, we also find that gene flow was reduced on fissioned relative to conserved chromosomes in a previously inferred, cross-karyotypic admixture event between *Cercopithecus cephus* ($2n = 66$) and *C. pogonias* ($2n = 72$). Although fissions may under some circumstances be selectively (nearly) neutral, this suggests that some of them indeed acted as barriers to gene flow, and thus potentially played a role during guenon speciation events. In search of specific genomic features that may explain the propensity of guenons to undergo chromosomal fission events, we inferred the repeat composition and structural variation across the genome and around chromosomal breakpoints. We found no genome-wide differences in repeat composition in guenons compared to their karyotypically stable sister lineage, Papionini, and chromosomal breakpoints were not generally enriched for specific repeats or structural variation. Consistent with previous cytogenetic studies, however, we found that most fissions occurred outside of centromeric regions. Since such non-centromeric fissions require the formation and activation of novel centromeres, this highlights centromere evolution in guenons as an intriguing prospect for future research. In summary, we provide insights into the genomics of the remarkable karyotypic diversity in guenons and its role in maintaining species boundaries, highlighting this primate lineage as a valuable system for studying chromosome evolution and speciation.



Genome evolution in holocentric organisms: insights from reconstructing the ancestral linkage groups of sedges (Cyperaceae)

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POSTER SESSION 2 - **P02.201**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

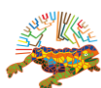
Holocentric organisms have centromeric activity distributed along the chromosome as opposed to concentrated at a single centromere. This has been hypothesised to permit high chromosome rearrangement rates relative to monocentric organisms. This is because fission of monocentric chromosomes typically results in one product lacking a centromere, preventing correct segregation during cell division, and fusion results in dicentric chromosomes with centromeres that may not be orientated in the same direction, creating an anaphase tug-of-war. In contrast, holocentric chromosomes will retain kinetochore activity on both fission products, and they have molecular mechanisms to coordinate the orientation of kinetochores, stabilising fusions.

Holocentric chromosomes are found in a wide range of organisms, including nematodes, many orders of arthropods, and several families of algae and vascular plants. However, direct evidence for a link between holocentricity and high rates of chromosome rearrangements remains lacking. Sedges are plants in the family Cyperaceae and possess highly variable karyotypes among extant species, with a haploid number ranging from 2 to at least 105, as well as holocentric chromosomes. The recent availability of high-quality chromosome-level sedge genomes makes studying the dynamics of chromosome change in this group possible; they represent an excellent system for the comparative analysis of chromosome evolution in holocentric species.

To this end, we used algorithmic approaches to reconstruct the ancestral linkage groups of sedges for the first time. Despite using over twenty chromosome-level genome

assemblies, inferring the history of chromosome evolution in the sedges was not straightforward given that sedge genomes are highly rearranged. For example, the algorithm of syngaph predicted an ancestral karyotype of $n = 1$ for the sedges' last common ancestor, which appears to be an artefact stemming from the influence of the very small karyotypes of Rhynchospora. The tool Agora provided an estimate of $n = 8$, more aligned with our prior expectations, but the output was sensitive to the removal of certain tips. Evaluating which algorithms and modules work best for reconstructing the ancestral linkage groups of sedges has important applications in the study of chromosome evolution in many other groups with high rearrangement rates, such as spiders.

These reconstructions also allow us to answer interesting questions regarding the biology of holocentric organisms. For example, are certain chromosomes more likely to fuse than others? Are chromosomal fission points reused, and if so, what features do these breakpoints share? Our reconstructions indicate that there have been on average more than 100 rearrangement events between the base of the sedge phylogeny and each tip, providing in total great statistical power to associate these events with hypothesised relevant genomic characteristics like chromosome length and GC content. Furthermore, using phylogenetically-aware methods such as path analysis and Bayesian models of correlated evolution we can ask whether rearrangement rate is associated with organismal traits ranging from genome size to ecology. The initial results of these analyses will be presented here.



Genome Structure Evolution in Ithomiini Butterflies

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POSTER SESSION 2 - P02.187, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Recent advancements in high-throughput, chromosome-level genome assemblies have uncovered striking variations in genome size and karyotype across organisms and allow large-scale investigations of the importance of genome structure variation as a source of diversity and its role in speciation. Chromosome-level structural variations, such as fusions, fissions, inversions, and translocations, can directly disrupt meiosis, reducing hybrid fitness and impeding gene flow. Additionally, changes in linkage disequilibrium between loci can alter patterns of genomic diversity, affect the efficacy of selection, and help preserve coadapted gene complexes, indirectly promoting reproductive isolation and speciation. However, the relative importance and generality of these direct and indirect effects remain poorly understood.

Lepidoptera (butterflies and moths) have holocentric chromosomes, female achiasmy, and a relatively conserved karyotype with a mode of 31 chromosomes. However, several lineages within the order show evidence of extensive chromosomal rearrangements. Among these, the Ithomiini tribe, a diverse and ecologically significant group of Neotropical butterflies, exhibits extraordinary karyotypic diversity, with haploid chromosome numbers ranging from $n = 5$ to $n = 120$ (Brown et al. 2004). This group is taxonomically rich and plays a vital role in mimicry rings, chemical ecology and tropical biodiversity. Their rapid radiation, with several genera exhibiting recent explosive diversification, makes them a powerful model for investigating the link between structural genomic evolution and speciation. Here we focus on characterising the genome architecture and quantifying the extent of the rearrangements in 200 species across the Ithomiini phylogeny.

Materials and Methods: In collaboration with international partners, we collected and flash-froze Ithomiini specimens from Central and South America. Genomic data were generated using PacBio HiFi long-read sequencing, Arima Hi-C and Illumina short-read sequencing for chromatin conformation capture, and

RNA-seq for transcriptome profiling. Chromosome-level genomes were produced by the Assembly and Curation Teams at the Tree of Life Programme, Wellcome Sanger Institute, using the genome assembly pipeline.

Structural rearrangements were characterised using both whole-genome alignments and gene order analysis of single-copy orthologs. We also annotated and assessed the relationship between genome structure variation and genomic features such as transposable element and gene content, nucleotide composition, and heterozygosity.

Results: Preliminary results from a subset of assembled genomes show chromosome counts corresponding to the earlier described karyotype range, but reveal more extensive rearrangements mainly involving frequent chromosomal fissions and fusions. Genome sizes vary widely, from under 300 Mb to almost three-fold, mainly due to lineage-specific expansions of LINE2 transposable elements.

We found a positive correlation between genome size and chromosome number, suggesting a constraint on individual chromosome size, potentially driven by cellular or meiotic mechanisms. Several genomes exhibit heterozygous fusion/fission events, indicative of ongoing chromosomal instability. Notably, one individual showed complex rearrangement chains involving multiple chromosomes in both haplotypes. Sex chromosome architecture was particularly dynamic. We identified multiple sex-autosome fusions, including Z-autosome and W-autosome fusions, with many species exhibiting multiple sex chromosomes. Despite this variation, the ancestral lepidopteran Z-chromosome segment remained conserved across all species.

Conclusions: The Ithomiini lineage is undergoing continuous chromosomal rearrangements and very dynamic neosex-chromosome formation. The clade represents a remarkable system for further studies of the interplay between genomic features and chromosomal rearrangements and how genome structure dynamics can influence the rate of speciation.



Genome wide identification of polymorphic inversions in a wild bird population and their possible phenotypic and fitness consequences

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*POSTER SESSION 2 - **P02.197**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM*

Chromosomal inversions create blocs of segregating alleles by suppressing recombination in heterokaryotypes. If this suppression can benefit the fitness of the individuals by preserving advantageous allele combinations into advantageous haplotypes, it also comes with a fitness costs. Inversion reduces the ability to purge deleterious mutations, and rare crossovers cause structural problems in meiosis, leading to unbalanced and infertile gametic products. Despite these potential detrimental effects, inversions are widespread across taxa, suggesting a trade-off between their evolutionary benefits and costs. Here, we leverage a new, haplotype resolved and near telomere-to-telomere

assembly with extensive genetic data from a long term monitored population of Swiss barn owls (*Tyto alba*) to investigate the fitness consequences of chromosomal inversions. By combining long-read information for structural variant calling – from Oxford Nanopore Technologies (ONT) and PacBio HiFi – and resequencing data from more than 3000 individuals, we identify eight putative inversions segregating in the population. Using comprehensive phenotyping and fitness data collected in this long term monitored population, we examine how these inversions impact individuals and what mechanism could underly their maintenance in the populations.



Genomic architecture imposes common constraints on hybrid genome composition across replicated hybridization events in an intertidal goby

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POSTER SESSION 2 - **P02.178**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Hybridization between distinct lineages is a widespread phenomenon, and thus many organisms harbor the resulting hybrid genomes. Investigating the common principles that govern hybrid genome composition is therefore important for understanding how the current biodiversity is generated. One powerful approach to uncover such common rules is the use of multiple independent replicates, which allows us to distinguish evolutionary necessity from stochastic outcomes. However, progress in identifying generalizable patterns in hybrid genomes has been largely limited by the rarity of clearly independent hybrid replicates in natural systems, with additional challenges posed by the technical demands of whole-genome-scale analyses.

Materials and methods: We investigated *Chaenogobius annularis*, a Japanese intertidal goby that offers three independent replicates of hybridization: two geographically distinct hybrid zones between intraspecific lineages, and one additional lineage that originated from an ancient intraspecific hybridization event. We generated a chromosome-scale genome assembly and obtained low-coverage whole-genome sequencing data for 416 individuals from 22 populations across both hybrid

zones and surrounding areas. Whole-genome cline analysis was performed based on allele frequency estimates to detect genomic regions exhibiting steep clines in both hybrid zones. These regions were then compared with those found in the hybrid-origin lineage.

Results: Genomic regions with consistently steep clines were significantly more common than expected by chance, suggesting shared genomic mechanisms that maintain parental differentiation. These regions were particularly enriched on the short arms of acrocentric chromosomes, which were characterized by low recombination rate and high repeat density. Moreover, many of these steep-cline regions also exhibited genealogical stability in the older hybrid lineage.

Conclusions: Our results suggest that genome architecture—particularly structural features associated with short arms—imposes persistent constraints on hybrid genome composition, limiting admixture and maintaining genomic boundaries. These constraints appear to act across both spatially and temporally independent hybridization events in this species.



Genomic Architecture of the Major Histocompatibility Complex Genes in the Neotropical Midas Cichlid

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POSTER SESSION 2 - **P02.191**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Host-parasite interactions are a major driver of evolutionary change. Parasites impose strong selective pressures on their hosts increasing the diversity of immunological genes and maintaining high polymorphism. The immune genes of the Major Histocompatibility Complex (MHC) are a hallmark of parasite-mediated selection. MHC genes belong to a highly dynamic multigene family characterized by frequent gene duplication and loss, presence of pseudogenes, and chromosome reorganization. These evolutionary patterns result in considerable variation in MHC architecture and genome organization within and across taxa. Cichlid fish provide a compelling model to explore these processes. Remarkable MHC class IIB diversity has been evidenced in this group, and immune gene evolution could be related to their outstanding diversity and speciation rates. To investigate the genomic organization of MHC class IIB in this fish family, we compiled nucleotide and amino acid sequences (~200) from the well-studied Neotropical Midas cichlid *Amphilophus citrinellus*, and additional (~100) sequences of four other Neotropical and African cichlid species for which high quality genomes exist. With this data we performed BLAST (blastn and tblastn) searches against all the chromosome-level annotated reference genomes. We identified the genomic locations and copy

numbers for these species and found considerable variation among species. The Neotropical Midas cichlid has 17 MHC class IIB loci distributed on five different chromosomes; the Neotropical *Archocentrus centrarchus* has 10 loci in four different chromosomes; the African riverine tilapia *Oreochromis niloticus* has 27 loci across 7 chromosomes, the Lake Malawi *Maylandia zebra* has 6 loci in two chromosomes; and the Lake Tanganyika *Simochromis diagramma*, has 21 copies spread across 10 scaffolds. All species exhibit similar exon ranges (4 to 9). These results evidence large variation in MHC genomic organization among cichlid fish, suggesting a very dynamic evolution of this gene family. It still remains unexplored whether MHC genomic variation expands to the intra-species level. This study provides the first comprehensive map of MHC class IIB loci in cichlid fish, offering key insights into the genomic basis of immune function in this rapidly diversifying lineage. The presence of multiple gene loci, copies, and exon ranges, underscores the potential for a wide diversity of immune responses shaped by host-pathogen and host-microbiome interactions. These findings establish a foundation for future work examining the immunogenetic mechanisms underlying adaptation and speciation, enhancing our understanding of how immune system evolution contributes to biodiversity.



Genomic architecture of the self-incompatibility locus in the apple tree provides insights into the evolution of collaborative non-self-recognition

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POSTER SESSION 2 - P02.190, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: The self-incompatibility (SI) system in flowering plants is a major barrier to self-fertilization, promoting outcrossing and maintaining genetic diversity. In the apple tree (*Malus* spp. genus), SI is governed by a collaborative non-self-recognition system involving a single pistil-expressed gene (S-RNase) and multiple pollen-expressed genes (SFBBs), all located within a single genomic region: the S-locus. A functional self-incompatibility haplotype (or S-haplotype) consists of one S-RNase gene and a specific combination of SFBB genes that together determine the pollen and pistil specificity of the recognition system. While the molecular players have been identified, the fine-scale genomic organization of this region — including the structure, order, and synteny of SFBB genes — remains poorly characterized. More broadly, how the extreme polymorphism of this locus is generated and maintained over time is still unclear.

Material and methods: Such detailed investigations have long been hampered by the complex structure and high repetitive elements' content of the S-locus, which is difficult to resolve with short-read sequencing. Thanks to recent advances in long-read technologies, we can now generate high-quality genome assemblies that enable accurate reconstruction of highly polymorphic regions. In this study, we used 27 S-haplotypes from four wild and cultivated *Malus* species, public or newly sequenced, to investigate the genomic structure and evolutionary dynamics of the S-locus across a broad phylogenetic range. We focused on characterizing the size of the S-locus, its gene content, and synteny of the SFBB cluster across

haplotypes, identifying patterns of recombination, gene duplication, and gene conversion. Additionally, we employed Deep Learning to predict structural models of SFBB and S-RNase proteins, investigate their interactions and intrinsically disordered regions, and assess signatures of positive selection at interacting residues.

Results: Our results reveal strong signatures of long-term balancing selection acting at the S-haplotype level, maintaining specific combinations of S-RNase and SFBB gene families in terms of gene content and synteny, despite a high degree of structural variability. From an evolutionary perspective, our findings highlight the role of local gene duplications in expanding SFBB repertoires, and suggest that positive selection and gene conversion between haplotypes may facilitate the spread of novel recognition motifs. Furthermore, we suspect that transposable elements may contribute to the structural evolution of the S-locus, based on their non-random distribution and consistent associations between certain TE families and specific SFBB families.

Conclusions:

Together, these results demonstrate that the genomic architecture of the S-locus in *Malus* is both dynamic and constrained. This flexible structure might be a key feature of collaborative non-self-recognition systems, enabling them to escape evolutionary dead ends and persist over long timescales. Overall, this work highlights how high-resolution comparative genomics of recognition loci can shed light on the interplay between selection, structural variation, and gene family evolution in complex adaptive systems.



Impact of gene family expansions, gene flow and a recent TE burst on the evolution of a unique reproductive strategy in fishes

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POSTER SESSION 2 - P02.177, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Evolutionary innovations often involve complex suites of traits that lead to significant changes in life history traits like feeding, reproduction or locomotion. Such “adaptive breakthroughs” and can open up new pathways for evolutionary diversification by promoting adaptive divergence. A rich example of evolutionary innovations are the ricefishes of Sulawesi, an island radiation of small brackish- and freshwater fishes. They evolved diversity in many aspects, for example, trophic morphology, salinity tolerance, sex determination, and chromosomal numbers. Most remarkably, they have evolved a unique reproductive strategy known as 'pelvic brooding' in two distantly related lineages. Females in pelvic brooding species carry fertilized eggs attached to their bodies by

filaments until the larvae hatch. This is assumed to be an adaptation to open water habitats as carrying the eggs allows to be independent from suitable spawning substrate. Even though known for decades, we only begin to understand the morphological and genomic prerequisites and the evolution of this unique trait. We present the first reference genome of a pelvic brooding species and discuss how expansion of specific gene families potentially shaped the evolution of this unique reproductive strategy. Furthermore, we show that the genomes of Sulawesi ricefishes were impacted by a recent burst of transposable elements and gene flow and discuss this in the light of genome evolution in this group.



Inversions Associated with Local Genomic Structure in *Culex pipiens* Mosquitoes from Southern Spain

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POSTER SESSION 2 - P02.181, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Inversions and other structural variants can play a significant role in evolution. In various mosquito species that transmit malaria and other pathogens, genomic inversions have been shown to colocalize with loci related to important epidemiological traits, such as insecticide resistance and vector competence. However, they have been overlooked in the past in the case of *Culex pipiens*, a major West Nile virus vector.

Material and methods: We analyzed whole-genome sequencing data from 96 *Culex pipiens* individuals collected across five provinces in southwestern Spain. To assess population structure, we performed principal component analyses (PCA) both genome-wide and in a sliding-window framework to detect localized patterns of genetic differentiation. We examined linkage disequilibrium (LD) patterns across the genome to identify regions of elevated LD consistent with structural variants. Additionally, we used manta, a structural variant caller, to investigate potential inversion breakpoints.

Results: Although genome-wide analyses showed no population structure linked to geographic location or degree of urbanization, windowed PCA revealed a region approximately 14Mb large on chromosome 1 exhibiting clustering pattern consistent with an inversion polymorphism. These regions also showed elevated linkage disequilibrium, supporting the hypothesis of structural rearrangements. The clustering of the samples in this region appear to indicate a close link between the inversion and the sex determination region, which is also located on chromosome 1. We also identified a likely breakpoint near one end of this chromosome 1 region.

Conclusions: Given how structural variation can shape evolution of important epidemiologically traits, further analyses are being carried to determine other loci collocated on this region. These results suggest that genomic architecture may play a previously underappreciated role in shaping variation in *Culex pipiens*, a key vector of West Nile virus.



Investigating the role of indels in allele specific expression in insects

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POSTER SESSION 2 - P02.185, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Compared to single nucleotide polymorphisms (SNPs) we know little about the impact and evolution of structural variants (SVs). Through short read sequencing technologies, the next generation sequencing era championed SNPs as the commonly used molecular marker. However, the lack of genome wide resolution from short reads to phase complete genomes resulted in an inability to determine accurate genome wide estimates of SV diversity. This has limited our investigative ability in determining the range of impacts SVs could have at the molecular, phenotypic and fitness level. This further prevented us from understanding how these impacts could alter the evolutionary fate of SVs. Now however, with access to high quality long read sequencing technology and sequencing consortia such as the Darwin Tree of Life (DToL) and Project Psyche the process of exploring genome wide SV diversity has begun in earnest.

In this poster I present the pipeline I have generated to explore the relationship between insertion and deletion (indel) variants and allele specific gene expression within insects. This is done using reference and alternate haplotype assemblies in conjunction with RNA seq data all from the DToL project. I align the RNA seq reads to shared exons between both haplotypes. Using informative SNPs the aligned reads are phased to retain only those that can be split between the haplotypes. Using this raw count data generalised linear models are then used to investigate how the properties and locations of indels predict their cis-regulatory impacts on nearby genes. The overall aim of this pipeline is to empirically investigate the molecular impact of indels, with the wider aim of determining how this impact predicts their fitness effects, which in turn predicts their genomic distribution and evolutionary fates.



Layers of Adaptation: Transcriptomic divergence in the mantle tissue of *Littorina saxatilis*

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POSTER SESSION 2 - **P02.202**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Current climate change and decline in biodiversity have resulted in challenges for many marine organisms. Knowledge about how genomic variation links to phenotypic variation is crucial to understanding why and how organisms adapt to changes, and key to predicting how they can persist in a changing world. In particular, gene expression regulation serves as a key mediator linking genome to phenotype, and structural genomic variants such as chromosomal inversions may play an underappreciated role in shaping these expression patterns. *Littorina saxatilis*, the rough periwinkle, is a well-established model for studying ecological speciation and local adaptation, especially given the repeated emergence of two distinct ecotypes, the crab ecotype and wave ecotype, across multiple geographic regions. These ecotypes exhibit pronounced differences in shell morphology and behavior correlating with their respective microhabitats and selective pressures. Despite extensive research into their ecology and genomics, the molecular mechanisms underlying the phenotypic divergence of these ecotypes remain largely unstudied.

Here we investigate gene expression differences and relating them to chromosomal inversions, and phenotypes in ecotypes reared in a common garden setting. In this pilot study RNA-sequencing of 30 individuals revealed distinct gene expression profiles between

ecotypes, including genes involved in biomineralization pathways, as well as sex-specific expression differences. Preliminary results also show differences in differential expressions might be linked to inversions. These early findings suggest that ecotypic divergence is reflected at the gene expression level, and that sex-specific expression may play a role in adaptive traits. However, the current sample size limits robust testing of the relationship between gene expression and inversion genotype.

We propose to expand RNA sequencing to 200 individuals with known phenotypes, genotypes, and relatedness. This will allow us to explore how inversions may influence gene expression and contribute to local adaptation. Our approach will provide valuable insight into the molecular mechanisms behind rapid adaptation in natural populations and in one bay may test the hypothesis that parallel ecotypic phenotypes may emerge through either parallel genomic changes or convergent regulatory shifts affecting distinct nodes in the same expression network. Our findings will advance understanding of how structural genomic variation influences gene regulation and adaptive divergence. This research not only sheds light on the evolution of *L. saxatilis* but also offers broader insights into how species might persist in rapidly changing environments.



Microsynteny and functional relatedness: insights into eukaryotic genome evolution

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POSTER SESSION 2 - **P02.209**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Genome rearrangements have shaped eukaryotic evolution, leading to substantial variation in genome organization and limited gene order conservation across distant species. However, evidence from prokaryotic operons and eukaryotic gene clusters show that neighboring genes are often functionally related, suggesting a potential link between genetic proximity and functional association. The relationship between gene function and gene order conservation (microsynteny) remains largely unexplored in eukaryotic genomes. Specifically, to what extent are gene microsynteny evolutionarily maintained, and are they more likely to persist when involving functionally related genes? Are there any major differences in this association for particular functional categories or lineages?

Here, we present unpublished work systematically investigating the association between microsynteny and function conservation, and its evolutionary relevance across a diverse set of eukaryotic genomes. We quantify the functional similarity between adjacent genes using Gene Ontology (GO) similarity measures and find that adjacent genes are more functionally related than

expected, compared to random gene pairs. Then, leveraging the unprecedented wealth of publicly available genomes and a recent method that reconstructs gene order across the entire eukaryotic tree of life (edgeHOG), we systematically quantify the conservation of gene adjacencies of extant and ancestral genomes. This allows us to date the emergence of these adjacencies and assess the relationship between their age and potential functional coupling of the genes involved. We also characterise the relevance of inter-gene distance and transcriptional direction, explore the role of gene expression and regulatory elements in gene order conservation, and study variation among the main lineages of eukaryotes.

Our work contributes to better understanding general trends in eukaryotic genome organization—providing more insights into the subtle interplay between genome rearrangements and gene function. At a more applied level, a better characterization of the relationship between functional coupling and gene neighborhood will improve in-silico predictions of gene function.



Mutating the mutators: mutational processes acting on transposable elements

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POSTER SESSION 2 - **P02.179**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Transposable elements (TEs) are not only selfish genetic elements, but they also drive structural changes in genomic sequences and can produce notable effects on phenotypes. Many advancements in the understanding of TE biology have been made using short-read datasets and comparisons with single reference genomes. But because of the difficulty of mapping reads accurately to specific TE insertions, short-read analyses have mostly focused on presence/absence polymorphism and less so on the variation at shared TE insertions present in multiple individuals. We study the patterns of mutation on TE copies using alignments across complete genomes from more than

500 *Arabidopsis thaliana* accessions that cover the diversity of the species. We aim to understand how TEs decay upon insertion and which mutational forces affect their sequence evolution over time. Additionally, we study the evolution of recombination landscapes of the sequences surrounding TEs to address the question of the possible influence of TEs on reassortment of genetic variation. Finally, the genome has a demonstrated capacity to domesticate certain TE remnants into functional regulatory sequences. Using our data, we also aim to elucidate the mechanisms by which TEs can become functionally important in a genome.



Reconstruction of the human amylase locus reveals ancient duplications seeding modern-day variation

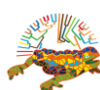
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POSTER SESSION 2 - **P02.194**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Starch digestion is crucial to human nutrition, with the amylase enzyme playing a key role in starch metabolism. The copy number of the human amylase gene is linked to metabolic diseases and adaptation to agricultural diets. Prior studies suggested recent origins of salivary amylase gene duplications. Here, we characterized 30 distinct amylase haplotypes across 98 individuals using long-read DNA sequencing and optical mapping. Phylogenetic reconstruction indicated that haplotypes with three AMY1 gene copies, prevalent across all continents and constituting about 70% of observed haplotypes, originated before the out-of-Africa migrations of modern humans. We further show that additional AMY1 gene copies existed in four archaic hominin genomes, suggesting that the initial duplication may have occurred up to 800,000 years ago. We analyzed 68 ancient human genomes from 300 to 45,000

years ago and found that AMY1 copy number variation existed long before agriculture (~10,000 years ago), indicating an adaptive increase in higher amylase copy numbers with the spread of agriculture. We identified the mutational mechanisms underpinning the diverse array of human amylase haplotypes, characterizing non-allelic homologous recombination (NAHR) and microhomology-mediated break-induced replication (MMBIR) as key processes. NAHR predominantly facilitates inversions and copy number variations within the locus, while MMBIR events underlie the observed AMY2 gene copy number increases. Our study provides a comprehensive understanding of the genomic structure of the amylase locus, identifying mechanisms and evolutionary history underlying its duplication and copy number variability in relation to the onset of agriculture.



Refining Sex Chromosome Strata Annotation Using X-Y Allelic Topologies

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POSTER SESSION 2 - **P02.171**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Sex chromosomes evolve through successive recombination arrests, forming evolutionary strata. Identifying these strata and their boundaries is crucial to understanding the evolutionary trajectories of X and Y chromosomes, but this task remains difficult—especially in young sex chromosome systems where divergence is low. In these cases, commonly used indices such as dS or FST tend to be noisy, and distinguishing ancestral from convergent strata requires combining several signals followed by gene-by-gene phylogenetic analyses. This motivated us to develop a new and more precise method. Our approach is based on the distribution of X-Y allelic topologies across the genome, inferred from SNPs identified using RNA-seq data and SEX-DETECTOR (a method detecting sex-linked markers based on segregation patterns within a family) and filtered using population data. For each gene, we calculate the proportion of observed topology types between species. These proportions reveal signals of recombination suppression: ancestral strata tend to group Xs with Xs and

Ys with Ys, while convergent strata show X-Y grouping within species. We use a newly developed changepoint analysis to detect transitions along chromosomes in these proportions and to define strata boundaries. The method was tested on both *Silene* and *Artemia*, where at least one species has previously described strata, allowing for validation of our method, while other species were so far unexplored. We compared our new method with strata annotation based on dS analysis and showed that our method is more robust and informative and allowed for finer delimitation of strata boundaries. In *Silene*, we found that all species share the same ancestral strata except for a small recent one specific to *S. latifolia*. In *Artemia*, we found one ancestral strata and several convergent strata. The method also avoids the need for predefined scenarios or gene trees, and works directly from SNP-level information, increasing statistical reliability. This new method is particularly suited for detecting recent strata in young sex chromosome systems and offers a direct way to visualize recombination history.



Repeat Driven Biases in Nanopore Sequencing Based Structural Variant Detection Reveal Clues to Chromosomal Rearrangements Linked to Infertility

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POSTER SESSION 2 - **P02.168**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Chromosomal rearrangements, including translocations, inversions, deletions, and duplications shape genome architecture and can impair fertility in carriers by disrupting meiotic pairing and reducing gamete viability. However, their detection remains challenging, particularly in repeat-rich genomic regions, where structural variant (SV) calling tools frequently report false positive break-ends (BNDs). Oxford Nanopore Technologies (ONT) long-read sequencing provides improved resolution in these complex regions, but the interpretability of SVs remains constrained by biases in detection algorithms. To address this, we developed a new SV detection pipeline designed to improve identification of complex variants—particularly reciprocal translocations—using pigs as a model for genome instability associated with reduced fertility.

Materials and Methods: DNA from three pigs (two with cytogenetically validated reciprocal translocations and one control) was sequenced using ONT and aligned to the reference genome with Minimap2. Two SV calling algorithms were used (Sniffles2 and BigClipper). For downstream analysis, only break-end calls were retained. Overlapping break-ends were defined as those with BNDs within 1 kb of each other and involving the same chromosome pairs in both tools' output. Benchmarking with Sniffles2 and BigClipper revealed substantial false-positive rates in repeat-dense regions. Permutation testing (RegioneR) was conducted to assess enrichment of SVs in repetitive elements. Repeat class-specific biases were further evaluated using the Genome Association Tester (GAT).

Results: ONT sequencing of the three pig samples yielded medium to high coverage (10–30X) with read N50 values between 5 and 16 kb. After down-sampling to 10X, Sniffles2 identified 162,344 SVs, including 4,329 BND events.

BigClipper, which detects BNDs by parsing CIGAR strings to capture large soft-clipping and alignment inconsistencies, identified 5,562 BNDs. Among these, 4,326 (Sniffles2) and 1,988 (BigClipper) were inter-chromosomal, suggestive of potential reciprocal translocations. A total of 299 inter-chromosomal BNDs overlapped between the tools. PCR validation confirmed the precise breakpoint of a validated chr9:chr18 translocation, demonstrating nucleotide-level accuracy. Hi-C validation identified a chr1:chr2 translocation breakpoint at ~100 kb resolution, which guided fine-mapping using the SV datasets to pinpoint the exact location. RegioneR analysis of overlapping BNDs detected by both tools showed significant enrichment in repetitive elements ($p = 0.001$, $Z = 3.269$), suggesting a bias toward repeat-rich regions. While this test applied to the full shared BND dataset, GAT analysis further revealed that false positives were particularly enriched in LTRs, satellites, and hAT transposons, whereas validated BNDs localized to underrepresented repeat classes including SINE-MIR, SINE-Pre0_SS, and LINE-L2d.

Conclusions: ONT long-read sequencing proved effective for detecting reciprocal translocations and refining breakpoint resolution, particularly when supported by PCR and Hi-C validation. However, inconsistencies between SV detection tools highlight the need for improved consensus strategies in complex, repeat-rich regions. Whilst findings reflect limitations in current algorithms, the repeated localization of validated breakpoints within specific transposable element classes suggests these elements may play mechanistic roles in mediating structural rearrangements. This work underscores the importance of refining SV detection pipelines to understand the link between genome architecture and infertility.



Sex-specific chromosome number and sex-determining mechanisms in the rhizocephalan *Peltogasterella gracilis* (Cirripedia: Rhizocephala: Peltogasterellidae)

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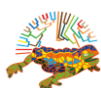
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POSTER SESSION 2 - **P02.169**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Barnacles (Crustacea: Thecostraca) exhibit diverse sexual systems, including hermaphroditism, androdioecy, and dioecy. While sex determination has been explored in androdioecious barnacles, the mechanisms underlying dioecy remain poorly understood. Among dioecious barnacles, kentrogonid rhizocephalans (parasitic barnacles) show striking sexual dimorphism: adult females develop root-like internal structures (internae) for nutrient absorption and prominent external reproductive organs (externae), whereas males are extremely reduced in size and reside within the female body. Notably, during early developmental stages, males are larger than females. Several rhizocephalan species exhibit seasonal variation in the offspring sex ratio, producing more females during summer and more males during winter. This pattern suggests that environmental cues may influence sex ratio variations. *Peltogasterella gracilis* (Rhizocephala: Peltogasterellidae), a parasite of *Pagurus* hermit crabs, has been considered as a model for studying sex determination in kentrogonid rhizocephalans. Previous research indicated that females with a diploid chromosome number of $2n = 30$ produce only male offspring, while those with $2n = 31$ (containing a univalent chromosome) produce only female offspring. However, the production of mixed-sex broods in autumn in *P. gracilis* appears to contradict the hypothesis that the presence of a maternal univalent chromosome solely determines the female types. To further investigate the

sex determination in this species, we examined the chromosome numbers of male and female offspring.

Hermit crabs (*Pagurus lanuginosus*) parasitized by *P. gracilis* were collected in Otaru, Hokkaido, Japan, between 2020 and 2023. Offspring sex was inferred based on the sizes of eggs or embryos. This was confirmed by the sex of cypris larvae of either the same or the previous brood, which exhibit clear morphological sexual dimorphism. Chromosomal analysis was performed using acetocarmine-stained squash preparations. Haploid chromosome counts were obtained during meiosis I in unfertilized eggs, and diploid counts were assessed during embryonic mitosis. All small eggs from female-producing externae had a haploid chromosome number of $n = 16$, while large eggs from male-producing externae consistently had $n = 15$. Among embryos, 97.4% of female embryos from female-producing externae exhibited $2n = 31$, while all male embryos from male-producing externae had $2n = 30$. These findings support the hypothesis that the univalent chromosome in females functions as a sex chromosome. We propose that *P. gracilis* employs a special form of female heterogamety (OW/OO system), which may facilitate seasonal regulation of offspring sex ratios. This system provides an adaptive mechanism by which genetic and environmental factors jointly determine sex ratio variation in this dioecious rhizocephalan.



Sloths Harbor the Highest Retrocopy Abundance Across Mammalian Genomes

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POSTER SESSION 2 - P02.193, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Retrocopies are a form of gene duplication in which germline-expressed mRNAs are reverse transcribed and reinserted into the genome as intronless copies. While most are non-functional, some can be retained, come under selection and evolve into new genes influencing species biology. Long Interspersed Nuclear Elements-1 (LINE-1) are the primary mediators of this process in mammals, driving retrocopy formation through their reverse transcription machinery. Here, we present chromosome-level genomes for two xenarthrans—the sloth *Choloepus didactylus* and the anteater *Tamandua tetradactyla*—and apply a standardized retrocopy annotation pipeline across mammals, now including Xenarthra, a basal placental lineage with unique physiological adaptations that was previously underrepresented in high-quality genome resources. **Methods:** we assembled the genomes with the Vertebrate Genomes Project v1.6 Pipeline, integrating Pacbio CLR, Bionano, Hi-C, Illumina short reads and manual curation. We applied our improved retrocopy identification pipeline (RCPedia, <https://www.rcpediadb.org>) to 41 mammalian genomes including the two newly assembled species and the publicly available *Bradypus torquatus*, *Choloepus hoffmanni* and *Dasypus novemcinctus*. **Results:** we report that both extant sloth genera, *Choloepus* and *Bradypus*, exhibit the highest number of retrocopies ever reported in mammals, with a 2.7-fold increase compared to other groups. Other Xenarthran species show considerably fewer retrocopies. Most sloth retrocopies are orthologous only within Pilosa (sloths), indicating a lineage-specific burst of retrotransposition in their last common ancestor. Using a curated repeat library to annotate all xenarthrans, we found a striking accumulation of young LINE-1 elements (Kimura divergence $\leq 10\%$) exclusively in sloths -absent or highly diverged in other Xenarthrans. Notably, more than other sloths, *C. didactylus* harbors thousands of full-

length copies from a handful of LINE-1 families, suggesting activity, and also presents the highest number of species-specific and total retrocopies in mammals. The overall retrocopy insertion pattern reflects what is observed in mammals: while most parental genes give rise to only one or two retrocopies, a subset undergo multiple retrotransposition events. In sloths, parental genes giving rise to retrocopies are predominantly housekeeping genes, and the characteristic out-of-the-X pattern is also observed, as in other mammals. The exceptional abundance of retrocopies in sloths inflates genome duplication estimates based on BUSCO scores, with 63% and 88% of the so-called duplicated BUSCOs overlapping annotated retrocopies in *C. didactylus* and *C. hoffmanni*, respectively. Finally, we searched for candidate domesticated retrocopies. We defined candidates as retrocopies with orthologs across sloths, an intact ORF $\geq 70\%$ the length of the parental protein, dN/dS < 0.5 , and expression in at least one tissue of *C. didactylus*. After manual curation, we identified 83 high-confidence retrocopies candidates. Many of these retrocopies are derived from parental genes involved in mitochondrial biology and metabolism, including TOMM20, MRPL34, HADHB, and CISD1. These findings are particularly intriguing given sloths' uniquely low basal metabolic rates—69% lower than other mammals in *Choloepus* and ~79% lower in *Bradypus*. **Conclusions:** Sloths harbor the largest accumulation of retrocopies amongst mammals and exhibit a high number of shared, lineage-specific LINE-1 families, many of which remain active, especially in *C. didactylus*. Although retrocopy insertions are typically neutral, they can also serve as raw material for evolutionary innovation. Our results suggest that sloths may have neo-functionalized retrocopies derived from parental genes involved in mitochondrial biology, potentially contributing to their unique metabolic adaptations.



Stepwise recombination suppression and degeneration in the mating-type chromosomes of two anther-smut fungi

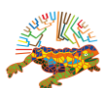
Boyer L¹, Ballu A¹, Rougemont Q¹, Lucotte E¹, Thierry M¹, Snirc A¹, Menezes A², Abbate J³, Hood M², Rodriguez de la Vega R¹, Giraud T¹

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POSTER SESSION 2 - **P02.200**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Recombination is beneficial in the long term to purge deleterious mutations and promote rapid adaptation. Recombination suppression nevertheless evolved repeatedly on sex chromosomes and mating-type chromosomes, often extending gradually with time, generating evolutionary strata, i.e., segments with distinct differentiation levels. Regions without recombination however degenerate due to less efficient selection, with the accumulation of deleterious mutations, transposable elements and rearrangements, and even gene losses. *Microbotryum* (anther-smut fungus) is a genus of basidiomycetous fungi that parasitize plants of the Caryophyllaceae family. Mating types are controlled by two loci with two alleles each, ancestrally located on two different chromosomes. In most *Microbotryum* species, the two mating-type chromosomes have fused and recombination has been suppressed between the two mating-type loci. As in sex chromosomes, multiple evolutionary strata (extensions of recombination suppression) occur in the mating-type chromosomes of *Microbotryum* species. The non-recombining region of mating-type chromosomes also displays signs of degeneration, with multiple rearrangements, the accumulation of transposable elements and deleterious mutations. In this study, we could obtain high-quality genomes of two

Microbotryum species with particularly degenerated mating-type chromosomes: *Microbotryum silenae-inflatae* and *M. violaceo-irregulare*. Both species are double haplo-lethal, meaning that haploid cells of either mating type are unable to grow, in contrast to other *Microbotryum* species. We studied the structure of the mating-type chromosomes and found three new evolutionary strata. In both species, we found more gene loss in the mating-type chromosomes than in autosomes and than in other *Microbotryum* species, and they were mainly located in the oldest evolutionary strata. Gene loss in mating-type chromosomes appears to occur mainly through rearrangements or to the insertion of transposable elements. Additionally, we identified in both species several genes lost in one of the two mating-type chromosomes but not the other (i.e., hemizygous genes), and not lost in other species able to grow at the haploid stage. These genes are predicted to code for essential functions. The hemizygous status of these essential genes could explain the haplo-lethal phenotype. In nature, the reproductive mode of *Microbotryum* (i.e., mating between products of a given meiosis) allow bypassing the haploid stage, sheltering these essential gene losses in a permanent diploid-like state.



Supergene polymorphism and its role in male morph differentiation in the bulb mite, *Rhizoglyphus robini*.

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¹Adam Mickiewicz University

POSTER SESSION 2 - **P02.175**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Sexual selection favours traits that aid reproductive competition, such as weapons or sexual ornaments. Understanding genetic underpinning of such traits is crucial for understanding the dynamics of sexual selection and its consequences for macroevolutionary processes. Here, we use whole genome re-sequencing to map genomic regions associated with expression of the sexually selected weapon in the bulb mite, *Rhizoglyphus robini*, which exhibits two distinct male morphs: armed fighters and unarmed scramblers. We identified a 3.5 Mb region on chromosome

seven that plays a crucial role in the male morph expression. This region exhibits characteristics typical of non-recombining haplotypes, including significant nucleotide divergence, recombination suppression, and enrichment of transposable elements. In addition to this region, the morph is influenced by many SNPs scattered across the genome. These SNPs are enriched from low frequency, possibly deleterious variants among scramblers. Our findings highlight the interplay between major loci of large effect and genome-wide variation, the latter likely shaping expression of SSTs via their effect on condition.



Telomere Position Effect and the Evolutionary Dynamics of Gene Expression Regulation

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POSTER SESSION 2 - P02.170, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The telomere position effect (TPE) represents an evolutionarily conserved mechanism by which chromatin architecture near telomeric regions modulates gene expression. Telomere shortening, a hallmark of cellular aging, leads to structural rearrangements in the genome that influence transcriptional profiles. This work explores the molecular evolution of TPE as a regulatory system shaped by selection pressures related to genome integrity and cellular senescence. We discuss how telomere erosion impacts gene regulation in aging cells, particularly in

the context of age-related pathologies, and highlight the evolutionary origins and diversification of telomere-associated chromatin remodeling pathways. Furthermore, we examine the relevance of TPE to evolutionary medicine, considering how variation in telomere biology may contribute to differential disease susceptibility and longevity across populations and species. These insights underscore the role of telomere dynamics in shaping organismal fitness and genomic stability over evolutionary timescales.



The effect of chromosomal inversions on gene expression in *Mimulus guttatus*

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POSTER SESSION 2 - P02.176, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

We comprehensively evaluate 64 inversions that segregate within a single natural population of the yellow monkeyflower (*Mimulus guttatus*). Nucleotide diversity patterns confirm low internal variation for the derived orientation (predicted by recent origin), elevated diversity between orientations (predicted by natural selection), and localized fluctuations (predicted by gene flux). Sequence divergence between orientations varies idiosyncratically by

position, not following the “suspension bridge pattern” predicted if the breakpoints are the targets of selection. Genetic variation in gene expression is not inflated close to inversion breakpoints but is clearly partitioned between orientations. Like sequence variation, the pattern of expression variation suggests that the capture of coadapted alleles is more important than the breakpoints for the fitness effects of inversions.



The formation and propagation of human Robertsonian chromosomes

de Lima L¹, Guarracino A², Koren S³, Potapova T¹, McKinney S¹, Rhie A³, Solar S³, Seidel C¹, Fagen B¹, Walenz B³, Bouffard G⁴, Brooks S⁴, Peterson M¹, Hall K¹, Crawford J³, Young A⁴, Pickett B³, Garrison E², Phillippy A³, Gerton J¹

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POSTER SESSION 2 - P02.208, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Robertsonian chromosomes (ROBs) are structurally variant chromosomes created by the fusion of two telo- or acrocentric chromosomes to make a single metacentric chromosome. First recognized in 1916 by Robertson in grasshopper karyotypes, these fusions are a common occurrence in nature, having since been recognized in many branches of life including plants, vertebrates, and invertebrates. In humans, ROBs occur in 1 out of 800 births, most commonly in female meiosis. The most common ROBs are the fusion of acrocentric chromosome 14 with either chromosome 13 or chromosome 21, suspected to form by a similar specific mechanism. In these fusions, the long arms are joined and parts of the short arms are lost. ROBs can occur de novo or be inherited. The recent complete assembly of the human CHM13 genome, including the short arms of the acrocentric chromosomes, followed by a population-level analysis of these regions for the first time, revealed Mb-sized homology blocks shared between acrocentric chromosomes, termed pseudo-homologous regions (PHRs). The existence of PHRs implies frequent interhomolog recombination and a working

model for how ROBs form. Herein we fully assemble three common ROBs: two 13;14 fusions and one 14;21 fusion. In all three cases, the ROBs have two centromere arrays, have lost all ribosomal 45S DNA repeats (or nucleolar organizer regions (NORs)), and are fused near a macrosatellite array composed of SST1 repeats. We demonstrate that the SST1 arrays on NOR-containing chromosomes bear hallmarks of exchange between chromosomes, including enrichment for PRDM9 DNA binding motifs which are associated with recombination hotspot activity. Further analysis of SST1 repeats and segmental duplications in human and nonhuman primate genomes suggests SST1 may have a broader role in genome rearrangements and evolution beyond Robertsonian fusions. Additionally, analysis of the two centromeric arrays on each ROB indicates epigenetic adaptations to support stable mitotic transmission, consistent with previous observations. In conclusion, we provide the first complete assemblies of common ROBs, precise mapping of their fusion sites, and novel insight into their formation and transmission mechanisms.



The genomic landscape of speciation in an Amazonian songbird

Mikkelsen E¹, Aleixo A², Weir J¹ Else Mikkelsen¹, Alexandre Aleixo², Jason Weir¹

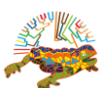
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POSTER SESSION 2 - **P02.198**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

As genomic data accumulates across the tree of life, common patterns in the genomics of speciation have begun to emerge. One emerging pattern is the repeatability of landscapes of genomic differentiation within clades. Genome scans in multiple bird lineages have suggested that genomic architecture plays an important role in shaping the landscape of differentiation during speciation, with the location of highly-differentiated loci often coinciding in related species pairs, but the drivers of these patterns remain unclear. To better understand the evolution of the avian genomic landscape we focused on a family of tropical songbirds: the manakins (Pipridae).

We sequenced whole genomes from four lineages of *Lepidothrix* manakins in the southeastern Amazon rainforest: *L. vilasboasi*, *L. nattereri*, *L. iris*, and *L. iris eucephala*. We use this genomic dataset to study the genomics of speciation in these shallowly diverged lineages. We find extensive gene flow between lineages that have come into contact (including evidence for selective sweeps proceeding across multiple species boundaries), providing a prime opportunity to compare how levels of introgression and differentiation vary across the genome. We find that differentiation amongst these species is primarily restricted to the sex chromosomes and narrow regions around the centromeres and telomeres. The

species possess few fixed differences in coding sequences, and those that do occur tend to be restricted to these pericentromeric or sex chromosome regions. By examining the landscape of differentiation across lineage pairs at different ages, we find that while most centromeric regions are highly differentiated between lineages that are at a more advanced stage of the speciation process (i.e., several hundred thousand years diverged), only a few are differentiated above an otherwise rather homogenous genomic background between populations at an early stage of divergence (i.e., less than 100,000 years diverged). To understand the extent to which these patterns observed in *Lepidothrix* manakins are repeatable, we generated differentiation landscapes for related groups of Amazonian manakins. Patterns were highly repeatable, with the greatest differentiation restricted to pericentromeric regions, telomeric regions, and the sex chromosomes. Patterns of diversity near the centromeres are indicative of recurrent selective sweeps driving the observed patterns of differentiation (suggestive of meiotic drive). Landscapes within the shallowest lineage pairs suggest that these sweeps occur rapidly but one-at-a-time, ultimately forming the conserved landscape of differentiation seen in these disparate manakin lineages.



The rise and fall of insecticide resistance: the role of genetic architecture

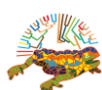
Caizergues A¹, Claret J², Ferber M¹, Milesi P², Labbé P¹

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POSTER SESSION 2 - **P02.207**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Adaptive genetic change is a major driver of species evolution. It can take various forms, ranging from simple single-nucleotide mutations to more complex alterations such as gene duplications. However, the impact of the genetic architecture underlying adaptive changes on their emergence and persistence within populations—especially under shifting selective pressures—remains underexplored. Insecticide resistance provides an ideal natural model to investigate this question, as it can result from both point mutations and copy number variations (CNV) in resistance genes. Moreover, it is driven by anthropogenic

selective pressure, the intensity, duration, and spatial extent of which are often well-documented. Using a long-term cline series of resistance genes in *Culex pipiens* mosquitoes, we examine how resistance alleles and gene copy numbers fluctuate both spatially and temporally, in response to insecticide use for mosquito control and its cessation in a Mediterranean region. Our study aims to understand how the genetic architecture of insecticide resistance influences the timing of genetic responses relative to the onset and withdrawal of selection pressure.



The role of gene duplications in speciation of Lake Baikal Amphipods

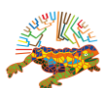
Korobitsyn I¹, Artiushin I², Fedorov D³, Bazykin G⁴, Burskaia V², Svardal H²

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POSTER SESSION 2 - **P02.213**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The adaptive radiation of amphipods in Lake Baikal has brought forward more than 340 species (20% of the world's freshwater amphipods), making it one of the largest lake species flocks in the world. In this project we study the impact of gene duplications on speciation of this group. Gene duplications are often a key to building new adaptations: neofunctionalized gene copies open a range of new metabolic and morphological opportunities. We prepared de novo long-read-based genome assemblies of two species, each of which represent independent invasions of amphipods into Lake Baikal. Although both of studied species have genome sizes of approximately

4Gb, it is known that genome sizes of the group vary in the wide range between 2Gb to 16Gb with constant chromosome number of 52. We hypothesize that multiple changes of genome size at the phylogenetic tree of the group are associated with independent gene duplication events and can shed light on the evolutionary history of the group. Based on new genome assemblies, we analyzed a list of duplicated gene families, which likely had an impact on explosive speciation of the group. Selection signatures indicate the most promising findings, which are associated with depth adaptation.



The understanding of structural complexity requires evolutionary context: Lessons from MHC architecture in Salamanders

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POSTER SESSION 2 - **P02.188**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Rapid advancements in sequencing technologies have transformed genomics—chromosomal-scale reference genomes are now becoming available across the tree of life. Yet, genome assembly is only the beginning; meaningful analyses remain challenging, especially in structurally complex and repetitive regions. A prime example is the Major Histocompatibility Complex (MHC), a gene-dense and evolutionarily dynamic region named after the key molecules it encodes: the bona fide MHC genes, a cornerstone of adaptive immune response of jawed vertebrates.

Given its structural complexity, high polymorphism, and rapid gene duplication and loss, the MHC is notoriously difficult to assemble and annotate. At the same time, a well-resolved MHC can attest to the high quality of a newly assembled genome and is often reported to complement genome analyses. However, if annotation and analysis does not fully consider specific evolutionary dynamics of the MHC region, they can mislead conclusions about the processes shaping vertebrate adaptive immunity.

We present a case study illustrating such risks, using recently published, high-quality, gigantic genomes of two urodele amphibians—the axolotl, *Ambystoma mexicanum* (genome size ~32 Gb) and the Iberian ribbed newt, *Pleurodeles waltl* (~20 Gb). We show how crude MHC annotations accompanying these publications, focused on syntenic genes found in mammalian MHC region, could misguide evolutionary inferences regarding large-scale genomic rearrangements, gene family expansion, and co-evolution among immune genes. In particular, we demonstrate that, contrary to earlier suggestions, salamanders possess a relatively compact MHC architecture

- with adjacent MHC class I and II regions—rather than a eutherian-like configuration where these regions are separated by a large genomic distance.

Through careful re-annotation, we clarify these misunderstandings and explore the broader implications for comparative immunogenomics. We further expand this perspective by incorporating data from newly sequenced genomes of several newt species (*Lissotriton helveticus*, *L. vulgaris*, and *Triturus cristatus*). Amphibians, as basal tetrapods, occupy a position critical for the understanding of MHC evolution. To date, insights have primarily come from frogs and toads, while their sister clade—the salamanders, characterized by massive genomes—have been underrepresented. Our new data provide a much-needed complementary perspective. For example, we identified several polymorphic and highly expressed MHC class I genes located in close proximity to antigen processing genes (APGs)—molecules that assist MHC class I in its function. This finding challenges a previously proposed hypothesis for non-mammalian vertebrates, which assumed that tight linkage between APGs and MHC class I drives coevolution among interacting partners and, in turn, constrains MHC class I duplication. Our results suggest that this model may not hold in salamanders.

In summary, our findings highlight the importance of accounting for the unique features of complex genomic regions, such as the MHC, to ensure accurate annotation. This is essential for reliable biological inference and to prevent misinterpretations that can ripple across scientific fields. We therefore advocate for a collaborative, cross-disciplinary approach to genome annotation—one that integrates structural genomics with evolutionary insight.



Understanding the role and evolutionary dynamics of "dark matter of the genome" of the economically important beetle *Tenebrio molitor*

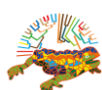
Šatović Vukšić E¹, Majcen P^{1,2}, Sermek A¹, Ugarković Đ¹, Oppert B³, Plohl M¹

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POSTER SESSION 2 - **P02.195**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The insect yellow mealworm, *Tenebrio molitor* has become an important model organism for various studies in biology, biotechnology and medicine. Expanding our knowledge about this organism is certainly a necessity following the European Commission's approval of *T. molitor* for human consumption. Repetitive DNA sequences, also called the "dark matter of the genome", are recognized more and more as key builders of genome architecture and drivers of its evolution. In line with that, transcription of tandemly repeated satellite DNA (satDNA) sequences was shown to be involved in numerous processes crucial for the cells. Notably, a substantial portion of the mealworm genome is composed of a single satDNA that accounts for approximately 50% of its total genetic material. Utilizing the Tandem Repeat Analyzer (TAREAN), we identified 10 additional satDNAs and examined their distribution within the mealworm genome. SatDNAs exhibit variations in terms of monomer grouping, array length, and chromosomal placement, with some being exclusively found on the Y chromosome. We also investigated the transcriptional activity of these satDNAs across different developmental stages (eggs, larvae, pupae, and adults) finding a significant increase in

transcription levels in male pupae and adults. Our NCBI blast searches across more than 3,000 insect genomes revealed the presence of these satDNAs in both closely and distantly related species. By juxtaposing the distribution of 11 satDNAs against the phylogenetic tree of insect species, we were able to estimate their evolutionary trajectories and determine their minimal age, which ranges from 310 to 380 million years ago. We have generated the divergence profiles of satDNA monomers, which provided insights into the processes of sequence turnover and homogenization for each satDNA. The mechanisms such as unequal crossing-over, transposition, and extrachromosomal circular DNA formation are considered as potential contributors to the spread of satDNAs within the genome. We have confirmed the existence of six satDNAs in the extrachromosomal circular DNA fraction, highlighting its role in the propagation of these sequences. In conclusion, our research lays the groundwork for a deeper understanding of the significance and evolutionary dynamics of the "dark matter of the genome" in this economically and ecologically significant insect, as well as in other species where these sequences are present.



Unravelling Sex Chromosome Evolution and Dosage Compensation Mechanisms in *Cameraria ohridella* (horse-chestnut leaf miner moth)

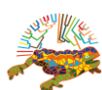
Bett V¹

¹Institute Of Science And Technology Austria

POSTER SESSION 2 - **P02.192**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Lepidoptera (butterflies and moths) exhibit female heterogamety (ZW system). While the Z chromosome is highly conserved across the clade, the origin, structure, and function of the W chromosome remain elusive. Intriguingly, basal Lepidoptera and their sister insect order, the Trichoptera, possess a ZO/ZZ system, suggesting that the W chromosome arose secondarily. However, the presence and functional role of the W chromosome are not consistent across lineages; some species show no evidence of its involvement in sex determination. Sex chromosome evolution often triggers dosage compensation (DC) mechanisms to balance gene expression between autosomes and sex chromosomes and consequently between sexes. ZW systems were traditionally thought to exhibit only partial DC, but recent studies in Lepidoptera, *Artemia franciscana*, and *Apalone spinifera* suggest a diverse range of compensation strategies, challenging traditional assumptions. While DC often involves chromatin-level regulation, the specific mechanisms in ZW systems are still poorly understood. To explore these questions, we generated a genome assembly of *Cameraria ohridella* (horse-chestnut leaf miner moth,

Gracillidae), and combined transcriptomic data from two tissues with CUT&Tag epigenomic profiling targeting both active (H4K16ac, H3K4me3) and repressive (H3K27me3) chromatin marks. This enabled us to characterize the composition and putative acquisition of the W chromosome in this species and to explore the functional contributions of its gene content. Our findings reveal a highly dynamic landscape of sex chromosome evolution in *C. ohridella*, including the ancestral Z (AncZ), a neoZ1 formed by fusion of AncZ with an autosome, and a neoZ2 that likely arose via formation of a neoW. These are accompanied by distinct dosage compensation patterns that differ not only between ancestral and neo-sex chromosome regions but also between tissues. Specifically, DC on AncZ likely involves repression of transcription in males (ZZ), while NeoZ regions show upregulation of gene expression in the heterogametic sex (ZW). Together, our study highlights the dynamic nature and mechanisms of dosage compensation in a species with secondary acquisition of the W chromosome and reveals how sex chromosome evolution can reshape regulatory landscapes in complex ways.



An episodic burst of massive genomic rearrangements and the origin of non-marine annelids

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MEETING ROOM 113, August 21, 2025, 2:00 PM - 5:00 PM

The genomic basis of cladogenesis and adaptive evolutionary change has intrigued biologists for decades. Here, we show that the tectonics of genome evolution in clitellates, a clade composed of most freshwater and all terrestrial species of the phylum Annelida, is characterised by extensive genome-wide scrambling that resulted in a massive loss of macrosynteny between marine annelids and clitellates. These massive rearrangements included the formation of putative neocentromeres with newly acquired transposable elements and preceded a further period of genome-wide reshaping events, potentially triggered by the loss of genes involved in genome stability and homeostasis of cell division. Notably,

while these rearrangements broke short-range interactions observed between Hox genes in marine annelids, they were reformed as long-range interactions in clitellates. Our findings reveal extensive genomic reshaping in clitellates at both the linear (2D) and three-dimensional (3D) levels, suggesting that, unlike in other animal lineages where synteny conservation constrains structural evolution, clitellates exhibit a remarkable tolerance for chromosomal rearrangements. Our study thus suggests that the genomic landscape of Clitellata resulted from a rare burst of genomic changes that ended a long period of stability that persists across large phylogenetic distances.



Aneuploidy generated by chromosomal rearrangements contributes to postzygotic isolation in butterfly hybrids

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Deviations from the typical karyotype of a species is generally referred to as aneuploidy and has been studied in model organisms such as humans, mice, worms and flies. Among whole-chromosome autosomal aneuploidies in humans, only trisomy 21 is viable into adulthood. *Drosophila* flies and *Caenorhabditis* worms can tolerate a larger fraction of their genome as aneuploid but generally not entire autosomes.

Outside of established model systems we know precious little about the generation, tolerance and consequences of aneuploidy. We here fill this knowledge gap by studying experimental hybrids between chromosomal races of wood white butterflies (*Leptidea sinapis*). This species shows an extreme range in intraspecific diploid chromosome number: from $2n = 57,58$ in Sweden to $2n = 106,108$ in Catalonia, Spain. We crossed these populations and produced both F_1 and F_2 hybrid offspring. We then whole-genome resequenced the entire pedigree of more than 220 individuals. By analyzing read-depth across the genome we found no aneuploidies in pure line individuals but aneuploidies in one F_1 hybrid and in dozens

of F_2 hybrids, with up to three aneuploidies in a single F_2 individual. These aneuploidies most likely arose in F_1 hybrid meiosis as a consequence of chromosomal rearrangements causing missegregation and the generation of unbalanced gametes. In particular, the results suggests that aneuploidies are especially likely to be generated by complex rearrangements involving more than three chromosomes forming multivalents in F_1 meiosis. Monosomy is significantly less tolerated than trisomy, in line with other animal systems. Intriguingly, F_2 females appear to tolerate trisomies better than F_2 males.

Our results suggest that the generation of aneuploidy may contribute to postzygotic reproductive isolation in this system by reducing the fitness in hybrid offspring. Even so, aneuploidy in this system is relatively well tolerated, perhaps due to the large number of chromosomes such that a single whole-chromosome aneuploidy only affects 1.5-2% of the genome. Our results shed light on the mechanistic basis of chromosomal hybrid sterility of relevance for the evolution of butterfly species and beyond.



Characterization of the transposable element landscape shaping the *Ectocarpus* genome

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Transposable elements (TEs) are mobile genetic units that play fundamental roles in evolution, yet their distribution and regulation remain understudied in many non-model eukaryotes. Brown algae, including the model species *Ectocarpus*, are only distantly related to animals, land plants, and fungi, from which they diverged over 1 billion years ago. They represent one of the most complex multicellular lineages on Earth. While significant progress has been made in understanding development, sex determination, and gene regulation in *Ectocarpus*, relatively little is known about mobile genetic elements in the species. This lack of knowledge extends across brown algae, and limits our understanding of how mobile elements have influenced the evolution of this major multicellular lineage.

Here, we combine manual TE curation with a recent chromosome-level assembly to characterize the *Ectocarpus* mobilome and examine TE regulation across its life cycle. We show that TEs occupy 28% of the genome, are mostly evolutionarily young, and feature a high diversity and abundance of DNA transposons. TE distribution is non-random: they are enriched in several repeat-rich intergenic regions, introns and on the sex chromosomes. Within the sex chromosomes, we hypothesize that ‘local

hopping’ of DNA transposons from the non-recombining sex determining region (SDR) to the pseudo-autosomal region (PAR) may contribute to the unexpected repeat accumulation in the latter.

Brown algal genomes lack DNA methylation and canonical repressive chromatin marks, such as H3K27me3 and H3K9me1/2/3, providing a valuable system for exploring alternative mechanisms of TE regulation. To investigate this, we generated and integrated RNA-seq, sRNA-seq, and ChIP-seq datasets across multiple life cycle stages. We find that intact and potentially active TEs are preferentially associated with 21-nt small RNAs and the non-canonical repressive histone mark H3K79me2. These repressive signals are largely stable across development, but show dynamic remodeling in gametes, implying a regulatory shift during the transition to the next generation.

Our findings highlight the complex interplay between TEs and the *Ectocarpus* genome across development, showing a non-random TE distribution, including enrichment of DNA transposons on sex chromosomes, and demonstrating that intact and potentially active TEs are preferentially associated with putative silencing mechanisms.



Conserved synteny and lineage-specific genome reshuffling in the largest animal order of Coleoptera

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While some animal lineages show highly conserved chromosomes, others show extensive chromosomal rearrangements, yet despite comprising nearly a quarter of all described animal species, chromosome evolution in beetles (Coleoptera) remains poorly understood. Consequently, it remains unclear whether genome restructuring has contributed to their exceptional diversification. Previous studies suggested that beetle genome architecture has remained largely conserved over 300 million years of evolution. More recently, nine ancestral linkage groups, termed Stevens elements, were proposed based on the *Tribolium castaneum* genome. Here, we revisit beetle genome structure incorporating significantly broader taxonomic sampling, utilising 210 chromosome-level genomes across the order. We redefine the ancestral coleopteran karyotype as consisting of eight elements and find that no extant species retains this ancestral configuration. Chromosomal fissions dominate rearrangement events, with autosome–autosome fusions occurring more frequently than X–autosome fusions. Despite this, the canonical X chromosome is conserved in almost all beetle species.

Breakpoint regions are often enriched in repetitive elements (particularly satellite repeats) and segmental duplications, suggesting a potential role in facilitating chromosomal rearrangements. Despite the remarkable ecological and morphological diversity of beetles, coleopteran genome evolution at the macrosyntenic level appears relatively constrained, with limited inter-element mixing following fusion events. Families exhibiting elevated rates of chromosomal reorganisation have evolved independently at least four times, partially coinciding with diversification bursts and the horizontal acquisition of genes associated with herbivory. Despite the overall chromosomal stability observed in most lineages, the microsyntenic gene order is highly shuffled. This reorganization appears to occur even in families without large-scale chromosomal rearrangements. Our results reveal a much greater dynamism in beetle genomes compared to other major insect orders and offer new insights into how lineage-specific constraints on genome architecture may have shaped their diversification in fundamentally different ways.



Cryptobiosis is associated with rapid chromosome restructuring in *Panagrolaimus* nematodes

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There is mounting evidence that genomic structure shapes evolution and adaptation to novel habitats. While morphological and genetic adaptations to environmental changes have been extensively studied, the impact of such changes on chromosome structure remains less explored. Recent advances in sequencing and genome assembly for non-model organisms are enabling detailed investigations of chromosome structure, revealing both conserved and highly rearranged patterns across animal phyla. In nematodes, 7 linkage groups of highly conserved elements, called Nigon elements, have been identified.

Cryptobiotic species can withstand extreme conditions in a state of suspended animation. This process may be expected to disrupt chromosome structure conservation by inducing DNA breaks and repairs. Few animal species have this ability, yet it is found in several nematode genera, such as *Panagrolaimus*. *Panagrolaimus* species are desiccation and freezing tolerant, enabling them to populate harsh environments such as the Atacama desert or the Siberian permafrost. This genus offers a unique opportunity to study the influence of ecological pressures on genome evolution.

We collected cryptobiotic *Panagrolaimus* species and closely related non-cryptobiotic *Panagrolaimidae* species to investigate the relationship between cryptobiosis and genome structure. Using Nanopore, PacBio HiFi and Hi-C reads, we generated 23 highly contiguous or chromosome-level collapsed and phased genome assemblies to analyze large-scale chromosomal rearrangements and conduct standard genomic comparisons.

We found extensive intra- and interchromosomal rearrangements between cryptobiotic *Panagrolaimus* species, several events of blunt chromosome fusion, and at least two events of triploidization. Further macrosyntenic analyses of phased assemblies reveal that these rearrangements and fusions are haplotype exclusive. These rearrangements may stem from DNA breaks induced by cryptobiosis that were not repaired to the original chromosome structure. Our results suggest a lenient genome repair machinery in *Panagrolaimus*, in contrast with the conservative system observed in cryptobiotic bdelloid rotifers. The flexibility of chromosome structure in *Panagrolaimus* is expected to be a driver of their ecological adaptation.



DNA on Repeat: Lineage-specific patterns of repetitive DNA accumulation across the genome size spectrum in Ferns, Gymnosperms, and Angiosperms

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Genome size varies dramatically across the tree of life, with plants showcasing some of the most extreme examples, including the largest known eukaryotic genomes. Nevertheless, we are still far from fully understanding how repetitive DNA contributes to genome size differences across major plant lineages. In this study, we present a large-scale comparative analysis of repetitive DNA composition among ferns, gymnosperms, and angiosperms—the three most species-rich clades of vascular plants—over a genome size range spanning from 0.06 Gbp/1C to 102 Gbp/1C. We have analyzed 340 genome skimming datasets using a modified version of the RepeatExplorer2 pipeline, tailored for large-scale repeat analysis, and integrated in TAREAN (Tandem Repeat Analyzer) to specifically identify conserved repeats. This has allowed us to robustly quantify, annotate and classify the main repetitive DNA sequences. Our results reveal distinct lineage-specific patterns in the accumulation and retention of repetitive elements (REs). Ferns, which generally have intermediate to exceptionally large genomes, show consistently high proportions of REs across all genome sizes. This suggests both sustained activity of repetitive elements and limited capacity for their removal, contributing to long-term genome expansion. Gymnosperms, despite their narrower genome size range, exhibit markedly lower proportions of conserved

repeats, implying extensive degradation and accumulation of genomic “dark matter.” This points to a different evolutionary trajectory characterized by low turnover and gradual repeat decay. Angiosperms, by contrast, as already found in previous studies show the broadest genome size distribution and the most dynamic RE landscape. From small to very large genome sizes (i.e. 0.06 - 149 Gbp/1C), angiosperms display both high and low RE content, driven by lineage-specific transposable element bursts and purging events, but in giant genomes (> 30 Gbp/1C) a shift is detected indicating a relative decrease in overall RE content. This contrasts with ferns, which retain high RE proportions even among the largest genomes. These contrasting trends reflect fundamental differences in genome evolution strategies across lineages. Our findings highlight repetitive elements as both agents and signatures of genome size evolution, shaped by lineage-specific constraints and mechanisms of genome maintenance. By leveraging genome skimming data and cross-clade comparisons with robust repeat analysis tools, this study provides a comprehensive overview of how repetitive DNA contributes to plant genome diversity. These insights expand our current understanding of genome evolution and offer a scalable framework for investigating genome size dynamics across the plant kingdom and beyond.



Do Newly Z-linked Sequences in Butterflies and Moths Show Evidence of Enhanced Protein Adaptation?

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Chromosomes present in a single copy in one sex often have unusual patterns of sequence evolution, as the effects of recessive mutations are not masked. Therefore, substitution rates may be elevated compared to the autosomes. In Lepidoptera, the Z chromosome is present in a single copy in females. As a result, it has been proposed that tortricid moths, which are agricultural pests, are evolutionarily successful due to accelerated protein evolution in newly Z-linked detoxifying enzymes (Yang et al., 2025). However, both “fast” and “slow” Z evolution have been reported in lepidopterans, depending on the species and genes examined. Whether an increase in the rate of evolution following a translocation to the Z chromosome is commonly seen across the taxon remains to be determined. In addition, it is not clear to what extent the amino acid changes observed are associated with shifts in protein function.

Using hundreds of lepidopteran genomes from the Darwin Tree of Life project, we ask: Is there evidence for accelerated evolution of

Z-linked sequences across lepidopterans? Are some genomic regions especially conducive to adaptation? Drawing on a catalogue of independent genomic rearrangements, we will explore a possible causal connection between becoming Z-linked and faster evolution, and how patterns differ between lineages and strata of different ages. This will reveal whether tortricids are unusual, perhaps on account of the characteristics of the autosomal segment that became fused to the Z chromosome, or if similar cases are widespread.

The dataset also provides an opportunity to examine if any observed acceleration is consistent with adaptation rather than drift. In addition to identifying fast-evolving proteins, pinpointing rapidly diverging sites will be informative. Past work on mammals reports that positively selected sites in xenobiotic-metabolising enzymes cluster around the active site. Considering the structural contexts of substitutions will, therefore, provide clues about functional consequences and adaptive potential.



Effects of hybridization on genomic content and expression of transposable elements in a hybrid-species complex

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Background: Transposable elements (TEs) are increasingly recognized as major drivers of eukaryote genome evolution. TEs replicate and insert themselves across the host genome which may lead to maladaptive effects but can also generate novel variation for selection to act upon. Due to this, hosts often evolve mechanisms to repress the TEs which then remain inactive in the genome. The effects of inter-specific hybridization on TE activity and the subsequent role of TEs in the evolution of hybrids are an interesting route by which evolutionary novelty may arise. The genomic shock hypothesis proposes that hybridization leads to a proliferation of TEs due to a mismatch between TEs and their repressors in hybrid genomes. Homoploid hybrid genomes are a mosaic of their parental genomes, and repressors which have co-evolved with TEs may be broken up. The effects of hybridization on TE activity have largely been explored with experimental first-generation crosses, but the long-term outcomes in natural hybrid species which have been evolutionarily successful is not well-studied. We investigated genomic content and expression of TEs in the stabilised homoploid hybrid species *Passer italiae* which originated through hybridization 5-6,000 years ago. We then compared this to its parental species, *Passer domesticus* and *Passer hispaniolensis*.

Materials and methods: We developed a bioinformatics pipeline to investigate genomic content and expression of TEs in the hybrid and parental species. We carried out annotation and analysis of TEs on genome assemblies of the three species. We estimated number of copies of a TE of a given family in the genome

indicating levels of past proliferation. Divergence from a putatively active consensus sequence was calculated (Kimura-2-parameter distance) for each copy. As inactivated TEs accumulate mutations, sequence completeness and divergence from the consensus sequence is used to infer the age of TE accumulation. We built a TE library including all TEs from our analyses combined with those present in Aves broadly. A *P. domesticus* genome was masked to include only TEs from this library. RNA-seq reads from testes and ovaries samples were mapped to this masked genome. We performed differential expression analysis between each pair of the three species. We investigated if TE expression in hybrids was transgressive (outside the range of the parent species).

Results: We found bursts of recent TE activity in the hybrid Italian sparrow for some TEs. These TEs show proliferation, consistent with expectations under a post-hybridization mobilization of TEs. The patterns of expression of active TEs differed among TEs, where some were similarly expressed in one parent and the hybrid, and some intermediate. As expected under a post-hybridization TE mobilization, 15.5% of TEs were transgressively expressed in testes and 6% in ovaries of the hybrids relative to the parents.

Conclusion: We provide here further evidence for the genomic shock hypothesis and for transgressive TE expression in a >5,000 year old hybrid species. Interesting future avenues for research are how genomic content and expression of TEs are linked, and how the mechanisms used by classes of TEs influences their activity post-hybridization.



Evolutionary dynamics of enlarged sex chromosomes and novel pseudoautosomal regions in Sylvioidea songbirds

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Sex chromosomes often include regions where recombination still occurs, known as pseudoautosomal regions (PARs). Understanding how these regions change over time is key to studying the evolution of sex chromosomes. In this study, we examine the dynamics of PARs in Sylvioidea songbirds, a clade with diverse neo-sex chromosomes formed by multiple fusions between sex chromosomes and autosomes. We show that the typically highly conserved ancestral songbird PAR has independently lost recombination multiple times in Sylvioidea. These losses occurred when new PARs emerged in the newly sex-linked regions. Interestingly, such PAR transitions were associated with fusions to the PAR-end, which occurred exclusively in some Sylvioidea lineages, but were actually driven

by later recombination suppression events rather than the fusions themselves. Moreover, by comparing male-to-female sequence divergence at PAR genes in species retaining the ancestral PAR, we provide support for the hypothesis that recombination suppression is preceded by sex-specific sequence divergence near the PAR boundary. This leads us to propose that PAR-end fusions have released the ancestral PAR from constraints in evolving recombination suppression and allowed PAR transitions along the neo-sex chromosomes. Overall, this study highlights remarkable diversity in PAR structure among birds and sheds new light on how recombination suppression evolves.



Frequent horizontal transfer of entire accessory chromosomes in fungi

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Horizontal chromosome transfer (HCT) of entire accessory chromosomes has been observed at low frequencies in certain pathogenic fungi. These non-essential chromosomes, that vary in presence among individuals of a species, can influence traits such as virulence and thus pathogenicity and host range. Yet, the frequency, mechanisms and consequences of their horizontal transfer remain poorly understood. Here, we studied HCT in the asexual entomopathogenic fungus *Metarhizium robertsii* and identified multiple independent transfers of a specific accessory chromosome between distinct strains during co-infection of an insect host. Each time, only the accessory chromosome and no other part of the genome was transferred. The recipient strain showed enhanced competitive fitness under certain host conditions, suggesting that the transferred chromosome carries traits that promote its persistence. Supporting this, the chromosome harbors genes associated with virulence and, surprisingly, genes potentially affecting chromatin conformation and chromosome segregation. The latter could

play a role in the specific transfer of the accessory chromosome, which could thus encode the factors for its own transmission. Phylogenetic analyses further revealed movement of the same accessory chromosome between *M. robertsii* and the related species *Metarhizium guizhouense*, demonstrating that HCT can occur across species boundaries. Moreover, we observed substantial deregulation of genes and transposable elements on the transferred chromosome in its new genomic context of the recipient strain, emphasizing the impact of the horizontal transfer. Frequent transfer events of this accessory chromosome were also detected during in vitro co-cultivation. These results highlight that HCT is more widespread and evolutionary impactful in eukaryotes than previously recognized, particularly in asexual organisms with limited conventional genetic exchange. While accessory chromosomes may enhance fitness depending on the environmental context, they may also act as selfish genetic elements, facilitating their own spread and contributing to genomic innovation – much like bacterial plasmids.



Genome scrambling in the fast-evolving chordate *Oikopleura dioica* questions our understanding of the evolution of genome architecture, challenging the concepts of species and reference genome.

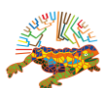
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Understanding how changes in genome architecture drive the evolution of biological functions, the innovation of novel adaptations, and ultimately, the generation of new species remains a central, yet unsolved, topic in Evolutionary Biology. Changes in genome architecture can include rearrangements of genes and their regulatory elements, as well as modifications of chromatin structure and epigenetic changes that affect topologically associated domains and 3D chromosome interactions. The general observation that genome architecture shows a high degree of conservation across relatively close species (or even distant ones after millions of years of divergence) had led to the assumption that strong evolutionary constraints limit variations in genome architecture. However, our recent discovery of an unprecedented amount of genome scrambling in *Oikopleura dioica* suggests that some of these evolutionary constraints may have been relaxed during the evolution of this fast-

evolving chordate, offering new perspectives on our understanding of genome architecture evolution. In this talk, we will present how genome scrambling affects the evolution of genome architecture in *O. dioica* across different specimens worldwide, unveiling the likely presence of multiple cryptic species despite the apparent absence of morphological differences. Results from various omics approaches begin to illuminate the extent to which this extraordinary scrambling can affect the evolution of biological functions. Finally, the likely presence of continuous variations in genome architecture across populations (or even within populations) of these cosmopolitan zooplanktonic animals challenges the notion of a single reference genome per species. Instead, it underscores the need to reinforce the concept of a pangenome that captures genome architecture changes across species variability.



Genomic rearrangements and the stepwise evolution of asexuality

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Transitions between sexual and asexual reproduction are rare and often evolutionarily constrained, yet some animal groups have undergone repeated shifts in reproductive mode. Planarian flatworms exhibit a striking diversity of sexual systems, including regular outcrossing, sperm-dependent parthenogenesis, occasional sex, and fissiparous asexuality via whole-body regeneration. Yet, how these transitions evolve remains poorly understood. Here, we study the planarian model species *Schmidtea mediterranea*, which exists as sexual and asexual strains, the latter linked to a conspicuous heteromorphic chromosome translocation hypothesized to have triggered asexuality. Using a highly contiguous, haplotype-phased genome assembly, we identify this as a balanced Robertsonian

translocation with a breakpoint in a large tandem-repeat gene desert—indicating no direct gene loss. However, beyond this visible rearrangement, we uncover pervasive chromosomal restructuring across all chromosomes. Genomic analyses of wild populations and laboratory strains reveal that these rearrangements are linked to elevated heterozygosity, indicating they act as barriers to recombination. Overall, our results suggest that successive genomic rearrangements in a sexual ancestor under inbreeding pressure rendered large portions of the genome functionally asexual prior to the shift to obligate fissiparous asexuality. Our findings support a model of gradual transition to asexuality that may help explain the extraordinary diversity of sexual systems in flatworms.



High centromere diversity is maintained across *Arabidopsis* genus

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Centromeres are an essential part of eukaryotic chromosomes, however, due to their complex repetitive structure in most species, little is known about their evolution. In contrast, *Arabidopsis thaliana* centromeres of different accessions were studied in detail. This knowledge gives an opportunity to study centromere evolution on a broader scale by expanding the focus of centromere research to a whole genus.

Here we use HiFi long-read genome assemblies to obtain full centromere sequences of a diversity of *Arabidopsis* species. This allows us to compare centromeres of *A. thaliana* with related species, diverged about 5 Mya.

Centromeres of *Arabidopsis thaliana* are formed of arrays of tandem repeats alternated with ATHILA transposons. *A. thaliana* centromeric repeats are highly chromosome-specific and centromere structures vary greatly between accessions. We found that while *A. thaliana* main centromere repeat type is CEN178, other

Arabidopsis species lack it, instead exhibit three other repeat types distinguishable by their length and sequence - pAa, pAge1 and pAge2. These types not only form whole centromeres in both of closely-related species, like *A. lyrata* and *A. halleri*, but are also present in endemic species, *A. cebennensis* and *A. pedemontana* that diverged from the rest of the genus about 1 Mya. Thus, all three types are functional and maintained on a larger timescale.

The diversity of centromeric transposons is also maintained genus-wide. Copia as well as ATHILA transposons are present in centromeres of all species.

However, some species show a different trend. *A. arenosa* is closely related to *A. lyrata* and *A. halleri*, and yet only one repeat type (pAa) is observed. Sharing of repeat monomers between chromosomes is also higher in this species, suggesting that repeat turnover process is not the same for different *Arabidopsis* species.



Holocentric Chromosome Dynamics: How Structural Rearrangements Shape Recombination and Drive Population Divergence in *Carex* (Cyperaceae)

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Background: Holocentric chromosomes are distinguished by the presence of diffuse centromeres, with kinetochore activity distributed along their entire length during cell division. In contrast to monocentric organisms—where chromosome fragments resulting from fissions are typically lost during cell division—holocentric chromosomes enable proper segregation and retention of chromosome fragments from fission. This characteristic facilitates extensive variation in chromosome number in holocentric lineages. Although chromosomal evolution in these organisms is thought to be a major driver of speciation, direct experimental evidence linking chromosomal rearrangements to speciation remains limited. Hybrids between different karyotypes often exhibit reduced fitness, potentially limiting gene flow and contributing to reproductive isolation. Moreover, chromosomal rearrangements can suppress recombination in genomic regions involved in local adaptation. Such suppression may further promote population differentiation and reinforce divergence between different karyotypes, ultimately contributing to the speciation process.

Material and methods: To investigate the role of chromosomal rearrangements in holocentric organisms, *Carex laevigata* was selected given its extensive inter- and even intrapopulation variation in chromosome number ($n = 39 + 11 - 42$). Three individuals from different populations, each with a distinct chromosome number (ranging from $n = 38$ to 40), were cultivated in a greenhouse for controlled crosses. F1 progeny was obtained through cross-fertilization between parental individuals (F1 hybrids were generated from two crosses: $n = 38 \times n = 40$ and $n = 39 \times n = 40$), and F2 progeny resulted from the self-fertilization of F1 individuals. Chromosome-level genome assemblies of the three parents were generated and compared to identify chromosomal rearrangements (fission/fusion

and reciprocal translocation) and structural variants (inversion). RADseq data from F2 individuals were analyzed to assess recombination patterns and investigate potential suppression of recombination due to structural heterozygosity. Finally, fitness and performance measurements and functional traits were collected to perform a QTL analysis, aiming to determine whether these traits correlate with rearranged regions of the genome.

Results: Comparative genomic analyses of the parental lines revealed multiple chromosomal rearrangements and structural variants among the parental genomes, including chromosomal fissions-fusions, reciprocal translocations, and inversions. By combining F2 RADseq data with high-quality genome assemblies of the parents, we detected pronounced reductions in recombination near the rearrangement breakpoints and in adjacent genomic regions. The QTL analysis revealed significant associations between measured performance and fitness proxies and several genetic markers in the parents.

Conclusions: Our study provides empirical support for the role of chromosomal rearrangements in shaping recombination landscapes in holocentric organisms. By combining chromosome-level assemblies of parental individuals with RADseq data from F2s, we demonstrated that structural heterozygosity in *Carex laevigata* leads to localized recombination suppression. Furthermore, QTL analysis revealed significant associations between fitness proxies and genetic markers in the parental populations. These results are consistent with the hypothesis that chromosomal rearrangements can influence patterns of gene flow and contribute to genomic divergence between chromosomal races in holocentric organisms.



Implications of Sex-Specific Genomic Differentiation in the Great Tit (*Parus major*) Species Complex

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The generation and exposure of genetic novelties to natural selection are central to evolutionary processes and sex-specific variation may function as a source of genomic adaptation. In birds—characterized by highly stable karyotypes and recombination landscapes—rapid evolution appears to occur under seemingly constrained genomic conditions. In this study, we performed comprehensive genomic analyses of more than 200 wild great tits (*Parus major*)—based on WGS and SNPchip data—from five Asian and seven European populations to uncover substantial cryptic and sex-specific genomic variation. We identified more than 50 autosomal genomic regions spanning over 70 Mb that harbor cryptic genetic elements. These gene-dense regions, which harbor genetic variation that is either female-specific or shared between sexes, show a significant overrepresentation of reproduction-associated genes. Using a

long-range deep sequencing approach, we discovered distinct haplotypes enriched in repetitive sequences with high GC and CpG content, which may stem from the sex chromosomes. These haplotypes contain hundreds of novel and divergent homologous genes under purifying selection, indicating functional conservation—particularly in pathways linked to female reproduction and development. We also address the technical implications of genome-wide sex differentiation, examining how sampling composition bias and SNP-based analytical limitations can skew inferences related to local adaptation, gene flow, and population structure. Our findings underscore the importance of rigorous quality control and sex-aware filtering strategies to account for these regions in population genetic analyses. At the same time, they demonstrate how sex-specific genomics can serve as a valuable tool for pinpointing genomic origins of rapid adaptation.



Non-canonical sex chromosome evolution revealed by extreme heterogeneity in homomorphic Y chromosome differentiation in the common frog

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MEETING ROOM 113, August 20, 2025, 10:30 AM - 12:30 PM

The canonical model of sex chromosome evolution states that sexually antagonistic selection drives recombination suppression, leading to gradual degeneration of the Y or W chromosome. While this model fits classical systems like humans and *Drosophila* flies, it fails to explain the widespread presence of homomorphic, non-degenerated sex chromosomes in many fishes, reptiles, and amphibians, where evidence for sexually antagonistic gene accumulation remains lacking.

The common frog (*Rana temporaria*) possesses homomorphic sex chromosomes with a male heterogametic system (XY males and XX females). Males of the common frog exhibit extreme heterochiasmy, with recombination largely confined to chromosome ends across the genome, whereas females recombine evenly across their chromosomes. Consequently, newly evolved sex-determining genes experience recombination suppression immediately, wherever on a Y chromosome they arise. We investigate sex chromosome differentiation at high resolution, using genome-wide Pool-seq, androgenesis and RNA-seq, in a previously studied Swiss population with striking within-population variation in Y

chromosome differentiation. Our results showed males with fully differentiated Y chromosomes (except the chromosomal ends), partially differentiated proto-Ys (only around the *Dmrt1* locus), or undifferentiated Y chromosomes (i.e., XX males). All types of Y chromosome show little evidence of degeneration: no major differences in gene expression, allele-specific expression, or transposable element accumulation, and no sign of gene loss. Only a subtle increase in non-synonymous substitutions and a recent burst of TEs were detected in the fully differentiated Y chromosome. Our results provide support that *Dmrt1* is the master sex determining gene, that there is a single origin of the Y haplotypes and of the "Fountain of Youth" hypothesis, where occasional X-Y recombination, likely via sex-reversed XY females, prevents Y degeneration despite suppressed recombination in males.

The results expand the scope of the canonical model and highlight an alternative pathway of sex chromosome evolution, in which the sex-determining gene rises within a pre-existing non-recombining region shaped by extreme heterochiasmy.



Polymorphic inversions distort selection scans: selection inference in the extremely complex genomic landscape of the spruce bark beetle (*Ips typographus*)

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¹Jagiellonian University

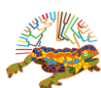
MEETING ROOM 113, August 21, 2025, 2:00 PM - 5:00 PM

Understanding the genetic basis of adaptation is a fundamental goal in evolutionary biology. Genomic selection scans have become powerful tools for detecting signatures of natural selection, but their application in genomes with extensive structural variation remains challenging. In particular, polymorphic inversions - chromosomal mutations that suppress recombination - can confound the detection of true selection signals. Inversion-rich genomes are emerging as hotspots of adaptation, but distinguishing true targets of selection from false positives in these regions is notoriously complex. Here, we investigate genome-wide patterns of selection in the European spruce bark beetle (*Ips typographus*), the most destructive forest pest in Europe, which harbours an exceptionally complex landscape of polymorphic inversions.

We analysed whole-genome resequencing data from 312 beetles sampled from 23 European populations, distinguishing two main geographical groups of northern and southern populations. Using two selection scan methods (InSL and Λ), we examined signatures of recent positive selection in datasets that varied in population composition and inversion content. Analyses were performed both on the whole genome and on subsets restricted to collinear regions or inversion homozygotes. We tested for inversion enrichment, compared overlap and divergence between scanning methods and datasets, and assessed the influence of inversion structure on selection inference. Finally, gene ontology (GO) enrichment analyses were used to test for overrepresentation of GOs in regions under selection.

We detected widespread signatures of selection across the genome, with strong enrichment of outlier signals within inversion regions. Inversions not only harboured more outliers than collinear regions but also exhibited longer and more clustered signals. We also observed strong selection signals outside inversions; some of them were not detected when inversions were included in the analysed data. Comparisons based on geographical groups of spruce bark beetle showed broadly similar selection landscapes between northern and southern groups but also revealed group-specific adaptive signals. Selection scans restricted to inversion homozygotes revealed haplotype-specific signals and filtered out potential false positives. GO analysis of haplotype-specific outliers identified functional categories related to environmental response and metabolic processes.

Our results highlight the dual role of chromosomal inversions as reservoirs of adaptive variation and sources of bias in selection scans. In *Ips typographus*, inversions are enriched for adaptive signals but may mask selection signatures elsewhere in the genome if not accounted for. By performing genome-wide scans using the inversion-rich genome of spruce bark beetle, we identify robust candidate loci under selection and demonstrate the importance of accounting for genome structure in evolutionary inference. This study provides one of the most detailed assessments to date of the impact of inversions on selection detection and provides a framework for future work in structurally complex genomes.



Recombination suppression through structural variation leads to sex chromosome evolution in the plant *Silene exscapa*

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MEETING ROOM 113, August 20, 2025, 10:30 AM - 12:30 PM

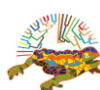
Appearance of separate sexes is usually associated with the evolution of sex chromosomes. Dioecy evolved many times independently in angiosperms providing an opportunity to study dynamics of their evolution. Sex chromosomes evolve from autosomes through recombination suppression around a sex-determining locus. However, despite many theories explaining why recombination might be suppressed, little is known about how this actually happens. In their early stages of evolution, sex chromosomes have small, weakly divergent non-recombining regions making them hard to detect. We here focus on *Silene exscapa*, a species that evolved separate sexes recently and ask if it has sex chromosomes and what their characteristics are.

We use a new haplotype-specific chromosome-level assembly of a female and

a male plant to map whole-genome resequencing data from three different populations, and look for sex-specific SNPs, coverage differences, and synteny between the haplotypes.

Amid a large amount of nucleotide and structural diversity, we detect a 1 Mbp long female-specific region on one of the chromosomes common to all three populations. It contains six W-specific genes without any Z counterpart.

In the newly discovered sex chromosomes of *Silene exscapa*, recombination suppression seems to start with an insertion or deletion, instead of an inversion as classically assumed. We discuss these findings in the light of genome and sex chromosome evolution.



Sex chromosome divergence and barriers to gene flow in brown algae with haploid–diploid life cycles

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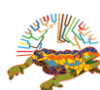
MEETING ROOM 113, August 20, 2025, 10:30 AM - 12:30 PM

Hybridization and genome divergence are central to understanding speciation, yet how structural genomic features influence postzygotic isolation remains poorly understood, especially outside classical model organisms. In this study, we investigate two closely related species of brown algae, *Ectocarpus siliculosus* and *Ectocarpus crouaniorum*, which have haploid–diploid life cycles and UV sex determination system. The two species were previously shown to form hybrid sporophytes in laboratory crosses and natural populations, however, recombinant gametophytes were nearly absent, suggesting a strong postzygotic barrier. By generating high-quality chromosome-scale genome assemblies for both species and sequencing individuals from natural populations including hybrid zones, we reveal frequent formation of F1 hybrids in the wild and uncover patterns of introgression consistent with rare backcrossing events, primarily with *E. siliculosus*.

Comparative genomic analyses reveal extensive divergence between the species, with the strongest differentiation on their sex chromosomes, particularly in the large pseudoautosomal regions (PARs).

Preliminary results suggest structural variation in the non-recombining region, including a potential inversion, and a notable enrichment of species-specific young genes in the PARs. Interestingly, the accumulation of species-specific genes on the PARs have been observed across many other brown algal lineages, may contribute to reproductive isolation and represent a general feature of brown algal sex chromosome evolution. Genome-wide introgression analyses and haplotype painting indicate largely uniform gene flow across the genome, without evidence for large introgression deserts, highlighting the potential role of the sex chromosome in driving species divergence and reproductive isolation.

This work provides insight into how sex chromosome architecture may mediate reproductive isolation in species with complex life cycles, and emphasizes the role of PARs as dynamic regions that accumulate lineage-specific innovation and divergence between species. This study offers new insights into how genome structure influences gene flow during the early stages of speciation in non-model, marine organisms.



Sex-specific recombination landscapes are associated with genomic and transcriptomic features in passerine birds

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MEETING ROOM 113, August 21, 2025, 10:30 AM - 12:30 PM

Heterochiasmy, the sex-specific variation in recombination rates and landscapes, is a widespread phenomenon across taxa that plays a crucial role in shaping genetic diversity and evolutionary dynamics. Yet, its genomic and functional drivers remain poorly understood. I will discuss emerging patterns of heterochiasmy in the house sparrow (*Passer domesticus*) and present preliminary results, highlighting associations between sex-specific recombination rates and genomic and transcriptomic features.

House sparrows offer a powerful system for studying the mechanistic basis of heterochiasmy, as they show stable sex-biased recombination patterns. Previous work has shown that the genome-wide crossover levels are female-biased in this species, but the chromosome-level recombination landscape may be either male or female-biased, resulting in distinctly male or female recombination hotspots. Despite these well-characterised patterns, their molecular drivers remain unknown.

To address this, we phased chromosomes from parent-offspring trios to generate individual-level crossover landscapes and integrated local genomic and transcriptomic information to uncover the mechanistic basis of sex-biased recombination. We show

that, at the chromosome-level, crossover events are more frequent in gene-dense, GC-rich, and telomeric regions, while being largely absent from centromeric and repeat-rich regions. We also observe that genomic regions with high crossover counts in females coincide with higher expression levels in chicken oocytes, suggesting a possible link between transcriptional activity and recombination in female meiosis.

To explore how conserved these patterns are across passerines, we compare these findings with the chromosomal recombination landscape of the helmeted honeyeater (*Lichenostomus melanops cassidix*), a critically endangered songbird. This species exhibits male-biased recombination, providing an opportunity to examine the potential evolutionary and mechanistic causes of heterochiasmy across different lineages.

By integrating multi-omic data in a comparative framework, we aim to identify genomic correlates of sex-specific recombination landscapes and contribute to a broader understanding of the molecular and evolutionary drivers shaping heterochiasmy in birds.



Signatures of local adaptation within chromosomal inversions

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MEETING ROOM 113, August 21, 2025, 2:00 PM - 5:00 PM

Chromosomal inversions are structural mutations where a chromosome segment breaks at two points and reinserts in the opposite direction, altering genomic architecture. Driven by advancements in comparative genomics revealing that chromosomes are more structurally fluid than ever thought, a revival of interest in chromosomal inversions can be observed concerning evolutionary processes ranging from mating strategies, through environmental adaptation, to speciation. Despite growing interest, how inversions drive adaptive evolution remains unclear. In heterozygotes, inversions suppress recombination, linking alleles in a non-recombining genomic block. Theory suggests that inversions aid local adaptation by preventing recombination between heterokaryotypes and protecting beneficial alleles from recombination. While ample empirical evidence is now linking inversion polymorphisms with local adaptation, we currently lack methods to identify selection targets within inversions. Coalescent theory has been used to derive expected patterns of divergence and diversity in simple scenarios, suggesting that it may be possible to identify targets of selection from sequence data. Here we build on these results and develop a simulation-based approach that allows us to characterize signatures of local adaptation within inversions in unprecedented detail.

To characterize signatures of local adaptation within inversions, we first ran forward genetic simulations of various evolutionary scenarios in SLiM across a range of parameters such as selection strength and migration rate. We then computed summary statistics from these simulations, including F_{st} , linkage

disequilibrium (LD), Tajima's D , and nucleotide diversity. We then tested the power of various outlier scans to identify parameter combinations and summary statistics that are the most informative. To ensure statistical robustness, we ran 50 replicates per scenario-parameter combination, allowing us to quantify variability and detect consistent signals of selection aiding adaptation across replicates. For that, we built a Snakemake pipeline that automated all workflow steps, from simulations through data processing to downstream analysis.

We describe signatures of local adaptation within inversions and identify the summary statistics that are most informative for characterizing molecular targets of selection. Additionally, we determine the parameter combinations under which outlier scans are most effective at detecting selected sites within inversions.

By integrating forward genetic simulations with downstream statistical analysis, we demonstrate that specific genomic signatures, particularly elevated F_{st} , high LD, and non-neutral Tajima's D , can be used to identify adaptive sites subject to selection within inversions. Our automated pipeline enables fast and scalable analysis of complex evolutionary scenarios involving inversions, representing a step forward in bridging theoretical models and analysis of genomic data from natural populations. This flexible pipeline and analysis of summary statistics lays groundwork for developing machine learning tools to detect selected sites within inversions.



Structural variation underlies rapid, repeatable adaptation during experimental evolution in a seed beetle

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MEETING ROOM 113, August 21, 2025, 2:00 PM - 5:00 PM

Background: Adaptation is the process by which populations become increasingly fit to an environment due to selection, but the types of mutations involved in adaptive evolution remain debated. Large-effect loci may promote rapid, repeatable evolution, while small-effect loci may reduce antagonistic pleiotropy. Given this uncertainty, two questions have emerged: 1) Is adaptation primarily accomplished using few large-effect loci or many small-effect loci? and 2) To what extent are the same loci used across independent populations?

Studies identifying the causal mutations underlying adaptation are rare, and fewer assess their repeatability. The driver of selection in natural populations is mostly unknown, and observations of truly replicated bouts of selection are uncommon. Non-adaptive genetic differentiation can obscure adaptive signals and short-read sequencing often fails to detect complex variation. Experimental evolution addresses these issues by subjecting controlled populations to known, replicable evolutionary pressures. Further, hybridization programs (i.e. backcross introgression lines) can parse signals of adaptive and nonadaptive differentiation. These methods were integrated with short- and long-read sequencing to identify adaptive genomic regions and evaluate their repeatability. This work combined replicated experimental evolution, short- and long-read sequencing, trait mapping, and a novel computational analysis using predicted 3D protein structure to determine the genetic architecture and repeatability of evolutionary rescue in a seed beetle.

Materials and methods: We induced host shifts in geographically diverse populations of *Callosobruchus maculatus* (cowpea seed beetle), from their native host to a challenging one (*Lens culinaris*, green lentil) to study the genetic architecture of repeated adaptation. From these lineages and their native-host ancestors, backcross introgression lines (BILs)

were created to separate adaptive from non-adaptive differentiation using three replicates from one population and one from two others. Pooled WGS sequencing from the adaptation experiment, GBS and long-read sequencing from the BILs, and trait mapping and RNAseq data from previous work were combined to elucidate the genomic basis of adaptation. A novel method used mutations identified in the WGS dataset to predict conformational stability of 3D protein structures (ΔG) unique to each lineage to identify potentially causal variants in regions of reduced recombination.

Results: All populations of *C. maculatus* adapted to lentil within 20 generations, and the BILs retained fitness on lentil despite introgression from the cowpea lineages. F_{st} in the WGS dataset showed that selection shaped genetic differentiation in a highly parallel manner in the adaptation experiment. Selection was more parallel within populations than it was between, with all populations showing variation on chromosome 1 but only the first (replicated) population exhibiting additional parallel differentiation signatures on chromosome 9. GBS of the BILs confirmed that variation in these regions was essential for survival on lentil, and nanopore sequencing identified a ~20MB structural variant on chromosome 1 in the region of elevated F_{st} . Trait mapping showed this region almost entirely explains genetic variation for adult mass and development time during adaptation, and the novel ΔG method independently identified an outlier gene within this region for which predicted protein stability segregates between pre- and post-adapted lineages. This gene contains motifs which suggest involvement in transcriptional regulation.

Conclusions: Overall, patterns of differentiation were highly parallel, the same large-effect structural variant was repeatedly used in adaptation, and adaptation may rely on highly pleiotropic transcriptional regulators



Understanding the evolutionary history of inversions and their role in speciation and adaptation across the *Littorina* marine snails genus

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MEETING ROOM 113, August 21, 2025, 2:00 PM - 5:00 PM

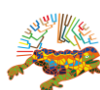
Background: How reproductive isolation barriers accumulate between nascent species remains a long-standing question in speciation research. Recently, chromosomal inversions have been put forward as good candidates to generate strong barriers to gene flow because of the reduced recombination rate between arrangements that help to maintain in high linkage disequilibrium genetic incompatibilities and/or locus under divergent selection. However, little is known about the time when inversions became established, and whether they can be re-used multiple times in the build-up of reproductive isolation in independent lineages.

Materials and methods: Here, we investigate the evolutionary history of inversions and their impact on speciation in 8 different species of *Littorina* marine snails, whose 2 (*L. saxatilis* and *L. fabalis*) are splitted into different ecotypes that have different shell phenotypes, behavior, and habitat preferences. We gathered 166 whole-

genome sequences from these 8 different species of *Littorina* and, when present, different ecotypes.

Results: We first confirmed the presence of more than 30 polymorphic inversions longer than 1 megabase pair separating species' ecotypes. We found complex histories of divergence, gene flux and contribution to genetic differentiation for all inversions. If the majority of it were species-specific, two of them were found in the same genomic region in different species. Within these inversions, we detected strong signals of shared polymorphism within arrangements between species, that can be explained either by introgression or ancient polymorphism sieved independently within each species.

Conclusions: We argue that this is a powerful system to improve our understanding of the evolutionary history of chromosomal inversions and their role in the evolution of barriers to gene flow in multiple closely related species.



What shapes the degeneration of neo-sex chromosomes across Lepidoptera?

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MEETING ROOM 113, August 20, 2025, 10:30 AM - 12:30 PM

In the absence of recombination, natural selection becomes less efficient, and non-recombining regions tend to degenerate over time, accumulating repetitive elements and losing functional genes. This phenomenon is particularly evident in the functional decay of centromeres and non-recombining sex chromosomes. The degeneration of non-recombining sex chromosomes has been widely studied in several taxa, including *Drosophila*, Lepidoptera, fish, and birds. However, a comprehensive understanding of the mechanisms and temporal dynamics driving this degeneration remains elusive. The causes of degeneration are still debated. It is unclear to what extent transposable elements and dosage compensation contribute to degeneration or if the observed degeneration is primarily driven by a reduction in effective population size (i.e. Hill-Robertson interference). More broadly, it is unclear whether the rate of degeneration varies across species, and which parameters best account for this variation.

To address these gaps, a comparative analysis of the rates of degeneration in non-recombining regions across species is needed. Recent sex-autosome chromosome fusions in achiasmatic species — in which recombination only happens in the homogametic sex — can provide insight into

the effect of recombination suppression. In Lepidoptera, where meiosis in female is typically achiasmatic, a neo-W chromosome is expected to experience a sudden arrest of recombination. Project Psyche is a pan-European collaborative effort to generate and utilise chromosome-level genomes of all 11,000 species of Lepidoptera found in Europe. As Project Psyche rapidly approaches its first goal of generating 1,000 genomes, this data provides a valuable opportunity to study the degeneration of recent neo-sex non-recombining regions.

As part of a preliminary analysis within the Psyche Consortium, we detected over 65 neo-sex chromosomes in these assemblies, with more than 20 found in female assemblies and recent enough to investigate degeneration. We quantified the extent of degeneration with the aim of answering the following question:

- How complete/irreversible is recombination arrest between neo-sex chromosomes?
- How does degeneration of neo-sex chromosomes depend on time since recombination arrest, the effective population size (N_e), and the size of the non-recombining region?

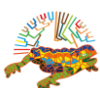




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S29 - Genomic Basis of Evolutionary Innovations



A High-Quality Reference Genome Assembly of *Oophaga pumilio*, the Strawberry Poison Frog

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POSTER SESSION 2 - **P02.222**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The strawberry poison frog (*Oophaga pumilio*) is a well-studied neotropical species known for its variable coloration, including aposematic and cryptic populations, toxicity, and complex parental care. These traits make it a valuable model for studying ecological and evolutionary processes, such as the interplay between natural and sexual selection. However, progress in understanding the genetic basis of these features has been limited by the absence of a high-quality reference genome.

The previously available *O. pumilio* draft genome, assembled from short reads, reflects the significant challenges presented by many amphibian genomes, namely large genome size (~6.7 Gb) and high repeat content (~70%), resulting in an extremely fragmented assembly. To overcome these limitations, we aimed to generate a chromosome-level assembly to support future research into the species' genetics, adaptation, and evolution.

We sequenced the genome of a captive-reared male *O. pumilio* using PacBio HiFi long-read technology (12X haploid coverage) and obtained Hi-C data from the same

individual to aid in scaffolding. Genome assembly was performed using hifiasm in Hi-C mode, followed by the Omni-C pipeline for chromatin conformation capture, scaffolding with YaHS and manual curation to achieve chromosome-level resolution.

The resulting contig-level assembly comprises 4,235 contigs, with an N50 of 14.97 Mb and a maximum contig length of 95.53 Mb. The estimated genome size is 6.98 Gb. Compared to the previous assembly, our genome is markedly more contiguous (191.9× larger N50) and more complete (BUSCO: 94.3% vs 76.6%). We also report a lower proportion of repeat elements (62.3% vs 70.4%).

In the next steps, we will perform gene annotation using TOGA and BRAKER2, supported by RNA-seq data from wild-caught individuals. Additionally, we aim to characterize the transposable element landscape of the *O. pumilio* genome.

This high-quality genome assembly provides a valuable resource for comparative genomics in anurans, particularly within the Dendrobatidae family.



ANTSR is an ancient sex-determining locus in bees

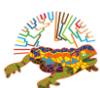
Rönneburg T¹, Taliadoros D¹, Everitt T¹, Olsson A¹, Magnusson S¹, Zetterberg Huser L¹, Martín-Hernández G¹, Bin Nor Fuad M¹, Cederberg B², Paxton R³, Seidelmann K³, Webster M¹

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POSTER SESSION 2 - P02.251, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Haplodiploid sex determination systems, in which females are diploid and males are haploid, are found in all species of Hymenoptera (wasps, bees, ants and sawflies). However, the molecular genetic mechanisms that specify sex are highly diverse and have only been discovered in a small number of species. In many haplodiploid taxa, the initial trigger is a complementary sex determination (CSD) locus, in which heterozygosity initiates a molecular pathway to generate females. In the honeybee *Apis mellifera*, the CSD locus overlaps an exon of a protein-coding gene and affects its amino acid sequence, whereas in the ant *Linepithema humile* it

affects expression of the lncRNA gene ANTSTR. Here, we map a single CSD locus in the genome of the red mason bee *Osmia bicornis*. This locus is homozygous in diploid males and exhibits extremely high levels of haplotype diversity. It is orthologous to the ANTSTR CSD locus identified in *L. humile*. We identify elevated levels of genetic diversity in the ortholog of the locus in other bee genera, suggesting that it may govern sex determination widely in bees. Our data are consistent with the hypothesis that ANTSTR evolved a role in sex determination at least 100 million years ago and is the ancestral sex-determination locus of bees and ants.



Assessing the phylogenetic position of sun spiders within chelicerates using complete genomes

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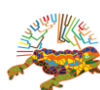
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POSTER SESSION 2 - **P02.252**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The phylogenetic relationships within chelicerates remain among the most controversial topics in arthropod systematics. This uncertainty is largely due to complex evolutionary events, including multiple independent whole genome duplications events. Furthermore, the inferred rapid radiations of terrestrial arachnid lineages might have obscured true signal in traditional phylogenetic analyses. Here, we present a phylogenomic study incorporating newly available genomic resources, including for the first time a chromosome-level genome assembly of a sun spider (Solifugae).

We annotated the genome of the Iberian sun spider *Gluvia dorsalis* and identified 15,217 protein-coding genes. The resulting high-quality proteome was used for phylogenetic

inference, initially restricted to the nine chelicerate orders for which complete genomes are available. We then expanded the dataset with proteomic data to include representatives from all 14 recognized orders. Additionally, we performed macrosynteny analyses to investigate patterns of chromosomal conservation and annotated Hox clusters to assess structural gene arrangements across taxa. Our phylogenetic analyses consistently recover Solifugae as the sister group to Acariformes, a relationship supported by both genome and proteome-based datasets. The assembled dataset will allow studying the genome history of the group and contribute to a more stable and resolved framework for chelicerate evolution.



Comparative genome analysis of giant clams and non-photosymbiotic mollusks

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*POSTER SESSION 2 - **P02.214**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM*

Giant clams (Tridacninae) are iconic in that they are the largest living bivalves. They rely on photosynthetic dinoflagellates (family Symbiodiniaceae) within their mantle tissues for much of their nutritional requirements. However, little is known about the molecular basis of this relationship and the genetic changes among giant clams that enable their photosymbiotic lifestyle. Here, we compared the genomes of six giant clam species with 17 non-photosymbiotic mollusk species,

representing a total of six molluscan classes. Giant clam-specific gene families were identified by orthology clustering and the top gene functions represented within these families were predicted using gene ontology enrichment. This study will provide insights on the establishment and maintenance of a functional giant clam holobiont and its impact on growth and responses to a changing marine environment.



Comparative Genomics of Syngnathiform Fishes: Linking Genome Evolution to Ecological and Phenotypic Diversity

Drewalowski J¹, Tensen L¹, Stiller J¹

¹University Of Copenhagen

POSTER SESSION 2 - **P02.232**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

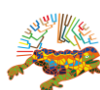
Syngnathiformes are an order of teleost fishes that includes seahorses, pipefishes and their relatives, which together present an exceptionally diverse and evolutionarily intriguing taxonomic group. They exhibit a remarkable array of morphological and life-history traits, such as male pregnancy, elongated bodies, and dermal body armor, some of which have evolved independently in different lineages. In addition to their phenotypic diversity, syngnathiformes are distributed globally and inhabit a broad range of ecological niches, spanning gradients in temperature, salinity, depth, and habitat types. This combination of ecological breadth and trait diversity makes them an ideal system for studying the genomic basis of adaptive diversification and evolutionary innovation.

To investigate the genomic foundations of this diversity, we have generated a densely sampled dataset of over 200 short-read-based draft genomes representing all nine syngnathiform families. Based on these assemblies, we characterized genomic traits such as genome size, repeat content, evolutionary rates, GC content and codon usage. We also reconstructed evolutionary relationships to investigate the phylogenetic signal across the genomes. This extensive dataset allows for robust comparative analyses that link genomic variation to key ecological and phenotypic traits.

Complementing the genomic data, we compiled an extensive trait dataset for each species, including ecological parameters, morphological features, and life-history characteristics.

Our dataset reveals enormous differences in genome size, varying by a factor of six among species. We identify multiple lineages that appear to have undergone genome expansion or reduction, largely driven by differences in the transposable element content. We present recent insights into the genomic correlates of key traits, including sensory systems. For instance, we document substantial variation in the opsin gene repertoire across syngnathiform families, highlighting differences that may reflect lineage-specific adaptations to diverse light environments.

More broadly, we explore correlations between gene family evolution and ecological or life-history traits, revealing patterns consistent with both convergent and divergent genomic responses to environmental pressures. Our findings shed new light on the genomic mechanisms underlying phenotypic diversification and adaptive evolution in Syngnathiformes. They also contribute to a broader understanding of how ecological opportunity and constraint shape genome evolution across deep timescales in vertebrates.



Conserved and Lineage-Specific Gene Regulation Underlie Daphnia's Fish Predation Responses: A Cross-Species Meta-Analysis

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POSTER SESSION 2 - **P02.244**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Phenotypic plasticity enables organisms to modify their phenotype in response to environmental change without underlying genetic variation. In aquatic ecosystems, Daphnia have become a textbook example of adaptive plasticity, particularly their inducible defenses against fish predators. Decades of ecological and morphological studies have documented extensive clonal and species-level variation in these predator-induced traits, yet the molecular mechanisms that translate kairomone perception into predator defenses—and the degree to which those mechanisms are evolutionarily conserved—remain poorly defined.

Understanding how these predator-response mechanisms function, vary, and evolve demands an integrative framework that integrates comparative genomics, transcriptomics, population genetics, and experimentally controlled exposures. Although recent 'omics work has begun to reveal candidate genes and pathways, each study often highlights dozens to hundreds of loci that may play a role in this plastic response—making it difficult to distinguish truly conserved, ecologically relevant signals from study-specific noise. A meta-study approach can distill this complexity by focusing on those genes and functions that repeatedly emerge across multiple species, experimental designs, and environments.

To that end, I performed a comparative transcriptomic meta-analysis across six Daphnia species (*D. magna*, *D. galeata*, *D. pulicaria*, *D. mitsukuri*, *D. sinensis*, *D. ambigua*) all exposed to long-term fish-kairomone treatments. Using a standardized RNA seq workflow, we first detected lineage specific differentially expressed genes and co expression networks. We then applied OrthoFinder to assemble these into orthologous groups shared by two or more species in response to predation signals. Functional annotation and enrichment analyses (GO, KEGG) then pinpointed the core molecular functions that are repeatedly mobilized in the predator response.

To connect plasticity to evolutionary change, we also re analyzed time series SNP data from a natural *D. magna* population sampled before and after fish introduction. Leveraging an updated reference genome and FST based scans, we assessed whether orthologous DEGs disproportionately fall within genomic regions showing elevated differentiation—thereby suggesting selection on plasticity related loci.

By integrating across species, study designs, and data types, this work aims to isolate the genes and pathways that are both conserved drivers of predator induced plasticity and targets of natural selection. Ongoing analyses will refine these candidates and ultimately test their causal roles using gene editing approaches in *D. magna*.



Convergent Recruitments of Venom Genes in Rear-Fanged Snakes

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POSTER SESSION 2 - **P02.253**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Venom is a trophic adaptation that has independently appeared within multiple groups in the animal kingdom. However, due to medical and epidemiological biases, toxinological studies have focused on a small array of taxa, leaving a large proportion of venomous species with little to no toxin-related data. Rear-fanged (opisthoglyphous) snakes possess a diverse arsenal of venom components whose evolutionary origins and diversification pathways remain underexplored compared to those of front-fanged species. Recently, this repertoire of toxins has been shown to include Snake venom metalloproteases (SVMPs), snake venom matrix metalloproteases (svMMPs), phospholipases A2 (PLAs2), C-type lectins (CTLs), Cysteine-rich secretory proteins (CRiSPs) among many other minor toxin classes. This study investigates the molecular mechanisms driving the origin and evolution of venom toxin families in rear-fanged snakes, as well as their patterns of divergence and convergence across the snake phylogeny.

Methodology: Sequencing the genomes of representative species of the family, and using a combination of syntenic analysis, venom gland transcriptomic data, venom proteomic evaluation, and phylogenetic reconstructions.

Results: Assembled genomes allowed us to identify varying degrees of interspecific collinearity across toxin loci. The SVMP, PLA2 and CTL loci showed a highly collinear scaffold in most species, with some exceptions for specific loci (e.g. the SVMP locus for *E. aesculapii* and the CTL locus for *H. carinicaudus*). On the other hand, the svMMP locus showed low levels of collinearity even within species of the same genus. SVMPs are the main proteolytic and hemorrhagic toxins in almost all venomous snakes, presenting different degrees of functional and structural diversification. In dipsadids however, we found that three independent tribes drifted away

from this SVMP dominance toward svMMPs, a completely different class of zinc dependent metalloproteinases. Moreover, svMMPs in these three tribes show similar structural variation to the one observed in SVMPs from vipers, converging toward more compact, simplified structures. On the other hand, PLAs2, which are commonly found in the venoms of vipers and elapids, are scarcely found as dominant venom components in rear-fanged species, except within the Pseudoboini tribe (Dipsadidae). We found that this tribe of snakes harbored a catalytically active PLA2 from the IIE subclass, which gives their venom similar in vitro functional properties to venom from vipers. In summary, although metalloproteinases and phospholipases A2 are almost ubiquitous in all families of venomous snakes, they appear to have been recruited a few times and from different gene sources to their venom glands. In another direction, the usually conserved CTL genes undergone strong diversification and amplification exclusively in Hydropsini tribe, likely becoming the key components of their venoms.

Conclusion: Our findings emphasize the dynamic and convergent nature of venom evolution across snake lineages, revealing that rear-fanged snakes exhibit complex molecular strategies akin to those of elapids and viperids. Nevertheless, while convergent at the functional biochemical level, venom evolution in rear-fanged species is shifting toward new, exclusive genes that have not been actively selected by other snakes. This work contributes to a broader understanding of toxin evolution and the ecological innovation of venom in opisthoglyphous snakes.

Financial Support: FAPESP and Fundação Butantan



De novo birth of transmembrane yeast proteins from intergenic polyA/T tracts

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POSTER SESSION 2 - **P02.219**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Intergenic genomic regions have essential regulatory and structural roles that impose constraints on their sequences, but they also have the potential to evolve into protein-coding genes. De novo gene birth, as this process is called, is now being recognized as a potent force for genetic novelty. In budding yeast, computational predictions have shown that intergenic regions harbor a higher-than-expected propensity to encode transmembrane domains, and this propensity seems to be linked to the high prevalence of predicted transmembrane domains in evolutionarily young de novo genes. However, what accounts for this enriched propensity is not known. Here we

show that specific arrangements of polyA/T tracts, which are abundant and enriched in yeast intergenic regions, explain this observation. We provide evidence that polyA/T tracts, which are known to act as Nucleosome Depleted Regions in a regulatory context, have been coopted through de novo gene emergence for the evolution of novel small genes encoding proteins with predicted transmembrane domains. Our findings contribute to our understanding of the process of de novo gene evolution and show how seemingly distinct but interacting levels of functionality can exist within the same genomic loci.



Decoding the evolutionary developmental genetic basis of novel animal signal traits

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POSTER SESSION 2 - **P02.256**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Acoustic courtship signals are crucial for mate attraction in many insect species – but how do developmental genetic programmes encode new signalling traits during evolution?

We tackle this question using the Hawaiian field cricket *Teleogryllus oceanicus*, in which a silent male morph, flatwing, arose under pressure from an acoustically-orienting parasitoid fly, *Ormia ochracea*. Silent males have feminised wing venation and reduced resonating structures, which correspondingly reduces or eliminates typical, loud, pure-tone signals. In the twenty years since flatwing was identified, multiple novel sound-altering wing morphs have arisen, some of which dramatically shift the acoustic characteristics of male calls.

We tested whether and how these morphological forms – which give rise to diverse new signal traits – are underpinned by rapidly evolving gene regulatory networks. Considering how frequently novel wing morphs are arising in this system, we hypothesise that a complex interplay of core developmental genes pattern sex-specific

wing venation as *T. oceanicus* wings develop, and slight adjustments to this network result in the alternative male wing venation which perturbs singing function.

To isolate the developmental genetic changes between flatwing and normal wing morphs, we first produced a timing schema of embryonic and nymphal development in both morphs, with key stages and instars characterised. Understanding developmental variation between morphs then enabled detailed spatio-temporal transcriptomic analysis and in situ hybridisation of wing development and venation genes. The doublesex transcription factor has emerged as a central player in flatwing-specific gene regulatory network divergence, although current genetic association evidence does not support a direct causal role via mutation. Thus, establishing upstream elements in wing venation networks is required to comprehensively link genotype to phenotype in this fascinating model of novel trait evolution in contemporary time, in nature.



Ecological divergence and retinal specialization in crater lake cichlids: a multiomic view of early sensory adaptation

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POSTER SESSION 2 - **P02.236**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

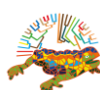
Crater lakes provide natural laboratories for studying sensory evolution in response to ecological gradients. In the African crater lake Barombi Mbo (Cameroon), endemic cichlids have diversified into distinct ecological niches across depth and light gradients. Previous studies using bulk RNA-seq showed that deep-water visual adaptation in these fishes often proceeds via shifts in opsin gene expression. However, this method obscures cellular and spatial dynamics that underlie early phenotypic divergence.

We integrate chromosome-level genomics, single-nucleus RNA sequencing (snRNA-seq), and fluorescence in situ hybridization (FISH) to examine retinal specialization in two recently diverged species: the deep-water *Myaka myaka* and the shallow-water *Pungu maclareni*. Both retain a full cone opsin gene repertoire, yet their expression profiles diverge strongly. *Myaka* shows high expression of green-sensitive RH2A α and RH2A β opsins in cones—likely enhancing spectral sensitivity under dim-light, mid-wavelength conditions. *Pungu*, by contrast, expresses long-wavelength (LWS) more prominently and, in a minor but at least detectable portion, UV-sensitive opsin, consistent with its broad-spectrum shallow

habitat. Our snRNA-seq results refine prior findings by detecting low-level LWS expression in *Myaka*, previously missed in bulk data, suggesting partial retention of red vision.

In addition, fluorescence in situ hybridization (FISH) reveals asymmetric RH2A opsin expression along the dorso-ventral axis in *Myaka*'s retina—an adaptation likely tuned to upward-scattered short-wavelength light. Interestingly, *Myaka* retains a square photoreceptor mosaic typical of shallow-water species, while another deep-dweller relative, *Konia dikume*, shows ventral clustering of single cones. This contrast hints at plasticity or incipient divergence: unlike *Konia*, *Myaka* undergoes seasonal depth migrations, moving to shallow waters during reproduction. The maintenance of broad spectral coverage and flexible retinal architecture may thus reflect a transitional visual strategy shaped by ecological fluctuation.

Our study highlights how regulatory shifts, cell-type resolution, and spatial patterning together illuminate the mechanisms of early-stage sensory divergence—offering a model for how ecological pressures shape complex phenotypes during adaptive radiation.



Evolution of chemosensory genes in the Chagas disease vectors of the genus *Rhodnius*

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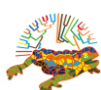
POSTER SESSION 2 - **P02.229**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The evolution of chemosensory genes has been studied among *Rhodnius* species, hematophagous bugs that are vectors of *Trypanosoma cruzi*, the causative agent of Chagas disease endemic in Latin America. Among *Rhodnius* species, some are domiciliary, meaning they invade anthropized environments in which they can be subjected to differentiated sensory stimuli. Chemosensory genes, namely Chemosensory Binding Proteins (CSPs), Odorant Binding Proteins (OBPs), Odorant Receptors (ORs), Gustatory Receptors (GRs), and Ionotropic Receptors (IRs), were annotated in 13 *Rhodnius* genomes, including those of four domiciliary species. Compared to genes already annotated in *R. prolixus*, 1 CSP, 1 OBP, 7 ORs, 1 GR, and 2 IRs were newly extracted from our data. For the well-assembled genomes (BUSCO > 80%), from

113 to 186 chemosensory genes were annotated according to the species.

Gene phylogenies confirm various expansion events for all multigene families. Tests for positive selection performed on well-defined orthologous groups within each family revealed 2 CSPs, 3 OBPs, 9 ORs, 3 GRs, and 13 IRs with sites under positive selection. Furthermore, positive selection was observed between the domiciliary species and others for an additional 4 ORs and 2 IRs. Purifying selection was also detected for GR1, a receptor homologous to the fructose receptor in *Drosophila*, suggesting the essential role of sugar for hematophagous *Rhodnius*.

These results suggest a rapid rate of evolution for these chemosensory multigene families and open the way to future development in vector control using chemosensory manipulation.



Exploring the genetic signatures of convergent evolution in termitophilous rove beetles

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POSTER SESSION 2 - **P02.230**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The ability to live in symbiosis with termites (termitophily) has evolved at least 40 different times independent of each other throughout several insect orders. It is especially prevalent within rove beetles (Aleocharine), where it has evolved tens of times convergently. The majority of termitophiles have evolved one of two different phenotypic adaptations to life inside a termite colony: Physogastry or Limuloidy. This enable the termitophile to evade an aggressive response from members of the host colony. This project aims to explore the genetic signatures of the convergent evolution of termitophily within Aleocharinae by means of comparative genomics, and use this group as a model system for the study of convergent evolution on a genomic scale. This includes genome assembly and comparative analysis of full genomes and transcriptomes of 38 Aleocharinae species including 10 free living and 28 termitophilous species covering approximately eight independent origins of termitophily. As most Aleocharinae are very small (only a few mm in size) and thus contain little DNA, PacBio HiFi long-reads

have been sequenced from 1-6 individuals per species. The HiFi reads have first been assembled for each specimen, and the assemblies of all specimens within each species will be combined into one consensus genome assembly per species using a custom pipeline currently in development that aligns each phased assembly and tiles the contigs into one consensus assembly. The consensus assemblies will subsequently be scaffolded to chromosome-level assemblies using OMNI-C chromatin capture sequencing data. Our preliminary HiFi-based genome assemblies suggest that Aleocharinae have relatively small genomes (150-350 Mbp) that are well below the average genome sizes of other Staphylinidae (app. 1 Gbp). In fact their genomes are amongst the smallest within Coleoptera which has an average genome size of 720 Mbp. With the annotated chromosome-level assemblies we plan next to test hypotheses that e.g. genome sizes, gene family sizes, and transposable element content evolved convergently in beetles with convergent termitophilous life strategy.



Genetic parallelism underpins convergent mimicry coloration across Lepidoptera

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POSTER SESSION 2 - P02.241, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

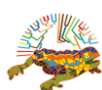
Background: Convergent evolution, the repeated emergence of similar phenotypes in response to shared selective pressures, is common in nature. One example comes from Neotropical Lepidoptera, where multiple chemically-defended species have evolved similar aposematic wing colour patterns to share the cost of predator education. While the genetic basis of Müllerian mimicry has been extensively studied in the *Heliconius* genus, it remains poorly studied across more deeply diverged lineages. Here, we investigate the extent of genetic parallelism and the roles of constraint and divergence time in shaping convergent wing colour patterns across Lepidoptera, spanning ~1–120 million years of divergence.

Materials and methods: We analyzed whole-genome resequencing data from 285 individuals, representing seven species from three lineages: Ithomiine and *Heliconius* butterflies, and day-flying Chetone moths. De-novo genome assemblies and genome-wide association studies (GWAS) were employed to identify colour patterning loci, before their functional roles were confirmed using CRISPR-Cas9 knockouts and HCR in-situ hybridization. Population and comparative genomics approaches were used to test for evidence of introgression and selection at these loci.

Results: In all species, the strongest associations with colour variation lie in non-coding regions near ivory and optix - highly conserved genes in wing colour patterning. Across multiple divergent species, GWAS signals for the forewing yellow bar are located in the first intron of ivory. Hindwing melanisation across

multiple divergent species is associated with distinct peaks near optix, likely corresponding to distinct cis-regulatory modules. CRISPR knockouts confirm the causal roles of ivory and optix. Despite evidence of genome-wide gene flow, we find little support for introgression or balancing selection at ivory and optix, suggesting that convergence occurs primarily through independent mutations, rather than through introgression or standing variation.

Conclusions: Our findings reveal that convergent wing color patterns across deeply diverged Lepidoptera consistently involve regulatory regions near ivory and optix, but not the repeated use of identical alleles. This gene reuse via independent mutations, rather than introgression or standing variation, highlights strong developmental constraints that channel adaptive changes along limited genetic routes. These results suggest that phenotypic convergence in the Neotropical tiger mimicry ring is driven by a simple and repeatable genetic architecture. The mimicry rings likely represent a series of locally adaptive fitness peaks, each accessible through a small number of large-effect mutations. This simple genomics architecture may make it easier for diverse taxa to evolve mimetic forms and join the ring. Once established, species may shift between mimicry patterns through regulatory changes. Together, our findings underscore the role of genetic constraints in driving parallel evolution and highlight the predictability of adaptation over deep evolutionary timescales.



Genomic mechanisms and evolution of a novel hemolymph neurotoxin in the Colorado Potato Beetle

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POSTER SESSION 2 - **P02.237**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

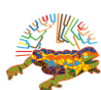
Background: Many insects harbour toxins in their hemolymph that are lethal to their predators, but not to themselves. However, the underlying genetic mechanisms and evolutionary drivers enabling toxin production and auto-resistance remain largely unknown. The Colorado potato beetle (*Leptinotarsa decemlineata*) is a notorious agricultural pest renowned for its remarkable resilience to environmental stresses and lack of natural enemies – traits likely linked to the production of leptinotarsin, a potent neurotoxin present in its hemolymph. Upon injection, leptinotarsin induces a sustained influx of calcium ions, triggering neurotransmitter release from presynaptic terminals and causing lethality in insects and vertebrates. Notably, leptinotarsin is present across all life stages, suggesting an essential but uncharacterized biological role. While its neurotoxic effects are well-documented, the genetic basis of leptinotarsin biosynthesis and the beetle's self-tolerance mechanisms remain unresolved, raising critical questions about how toxin-rich organisms evolve precise regulatory pathways to avoid autotoxicity.

Materials and methods: To address this, we combined transcriptomic, proteomic, and functional genomic approaches to identify leptinotarsin-associated genes. Hemolymph toxicity was first confirmed via injection assays in adult *Drosophila melanogaster*, establishing a robust bioassay system to quantify leptinotarsin's lethal effects. Toxic

hemolymph components were then isolated by fast protein liquid chromatography (FPLC), and characterized using shotgun proteomics. We established RNA interference (RNAi) feeding assays to target candidate genes, with subsequent injection of RNAi-treated beetle hemolymph into *Drosophila melanogaster* to confirm reduced lethality.

Results: Our findings challenge previous hypotheses suggesting leptinotarsin evolved from juvenile hormone esterase. Instead, we found novel candidate genes linked to toxin production. Contrary to earlier claims of hemolymph-based synthesis, tissue-specific expression analysis of candidate genes revealed fat body involvement in toxin production. Our preliminary results suggest a potential role of serpins, a conserved family of serine protease inhibitors, in leptinotarsin production and/or regulation. This parallels serpin-mediated protease inhibition in parasitoid venoms, where they suppress host immune responses, and aligns with their broader role in arthropod innate immunity.

Conclusion: Together, our work establishes the first genetic framework for leptinotarsin biosynthesis and advances our understanding of the evolutionary mechanisms of novel hemolymph toxins in insects.



Host use drives convergent evolution in clownfish

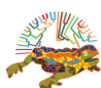
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POSTER SESSION 2 - P02.216, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Clownfishes (Amphiprioninae) are a fascinating example of a marine radiation. From a central Pacific ancestor, they quickly colonized the coral reefs of the Indo-Pacific and diversified independently on each side of the Indo-Australian Archipelago. Their association with sea anemones has been proposed to be a key innovation that enabled the clownfish radiation. However, this intuition has little empirical or theoretical support given our current knowledge of the group. To date, no ecological variable has been identified to explain clownfish niche partitioning, phenotypic evolution, species co-occurrence, and thus, the adaptive aspect of the group's radiation. Our study solves this long-standing mystery by testing the influence of sea anemone host use on phenotypic divergence. We provide a major revision of the known clownfish-sea anemone host associations, accounting for the biologically relevant aspects of host associations. We gathered whole-genome

data for all 28 clownfish species and reconstructed a fully supported species tree for the Amphiprioninae. Integrating this data into comparative genomic approaches, we demonstrate that the host sea anemones are the drivers of convergent evolution in clownfish color pattern and morphology. During the diversification of this group, clownfishes in different regions that associate with the same hosts have evolved similar phenotypes. Comparative genomics also reveals several genes under convergent positive selection linked to host specialization events. Our findings reveal that the sea anemone host plays a crucial role in driving clownfish diversification. This highlights how a strong mutualistic interaction can promote the diversification of entire clades by influencing their phenotypes, defining their geographic distribution, and ultimately contributing to their evolutionary and ecological success.



Incomplete Lineage Sorting and Its Role in the Recurrent Evolution of Parthenogenesis in Stick Insects

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POSTER SESSION 2 - **P02.234**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The repeated evolution of similar traits in related species occurs through diverse mechanisms, including the independent selection of shared ancestral variants in different lineages. This mechanism can cause incongruences between gene and species trees, known as incomplete lineage sorting (ILS). Parthenogenesis has evolved multiple times in stick insects, offering a unique opportunity to test whether ILS contributes to such parallel trait evolution. Here we study whether ILS contributed to the parallel evolution of parthenogenesis in *Timema* stick insects. As *Timema* species diverged up to 30 mya, mapping genomic data to a single reference genome can introduce biases and mapping errors due to high divergence. Therefore, we tested a “lifter” approach in which samples are mapped to a reference from their own species. Inferred genotypes are then assigned to the homologous region of a different species by converting genome coordinates using whole-genome alignments. Compared to the traditional “single reference” method, the “lifter” approach consistently improved mapping

rates in gene regions, especially for highly diverged species. To quantify the amount of incongruences between gene and species trees in *Timema*, we inferred topologies along the genome and found that ~10% of them differ from the species tree. ABBA-BABA tests revealed little evidence for introgression, suggesting that ILS is the primary source for phylogenetic incongruence in these taxa. To identify candidate regions for parthenogenesis in *Timema*, ongoing analyses are scanning for loci with excess shared variation between parthenogenetic lineages. By contrasting coalescence times along the genome, we will confirm whether candidate regions reflect ancestral polymorphisms (ILS; increased coalescence times) or introgression (recent coalescence times). Altogether, this work sheds light on the evolutionary dynamics underlying the repeated evolution of similar traits in highly diverged lineages and highlights the importance of ILS in shaping genomic patterns over long-term evolutionary trajectories.



Investigating the Cellular and Molecular Basis of Regeneration

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POSTER SESSION 2 - P02.220, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Regeneration is a well-known process in developmental biology and has been extensively studied across a wide range of animals, as it is key to understanding the evolution of Metazoa. In annelids, or segmented worms, the ability to replace lost body parts is both important and widespread. However, regenerative capabilities vary greatly among species, ranging from complete restoration of both anterior and posterior ends to more limited tissue repair. One of the main open questions in this field is the cellular origin of the newly formed tissues. Whether regeneration depends on pluripotent stem cells, dedifferentiated mature cells, or lineage-restricted progenitors remains a central challenge in annelid regeneration research.

Materials and Methods: In our group, we have approached this question by investigating the transcriptomic landscape of regeneration in species such as *Pristina leidy* and *Ophryotrocha labronica*, two species that exhibit distinct regenerative and reproductive capabilities. We focused on identifying key regulatory genes, as well as those linked to cell pluripotency, stem cells, and cell proliferation, which may drive the regenerative process in both species. To further explore these cellular mechanisms, along with the molecular dynamics and regulatory patterns during regeneration, we used single-cell RNA sequencing (scRNA-seq) during the anterior regeneration of the annelid *P. leidy*. A morphological staging system was developed to select the most relevant timepoints for sampling. The scRNA-seq data were then analyzed to define cell types, trace differentiation trajectories, and identify gene regulatory networks involved in regeneration.

Results: Bulk transcriptomic analyses revealed the activation of genes related to stemness, pluripotency, and cell proliferation in both species, despite their different regenerative capacities. Notably, genes associated with pluripotency and proliferation were conserved and differentially expressed during regeneration. The scRNA-seq analysis in *P. leidy* identified distinct cell populations, including proliferative stem-like cells, transient progenitors, and differentiating cell types contributing to tissue regeneration. Trajectory inference revealed dynamic changes in gene expression and lineage progression during anterior regeneration. We also observed the reactivation of stem cell-associated transcriptional programs in multiple cell populations. Moreover, comparison with posterior regeneration data allowed us to assess whether the same cellular and molecular mechanisms are deployed in different regenerative contexts. Some signatures were shared, while others appeared to be specific to anterior regeneration.

Conclusions: Our results highlight the complex and dynamic nature of annelid regeneration at the cellular and molecular level. The presence of conserved gene expression programs and stem-like populations suggests that common mechanisms may underlie different types of regeneration in annelids. The combination of bulk and single-cell transcriptomics offers a powerful framework to better understand the cellular basis of regeneration and provides new insights into the evolution of regenerative mechanisms in segmented worms.



Lineage-specific diversification of AluSg and AluSx3-derived alternative splicing events of exonization in RPS29 gene during primate evolution

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POSTER SESSION 2 - **P02.217**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Ribosomes consist of a small 40S subunit and a large 60S subunit and catalyze protein synthesis. RPS29 (40S Ribosomal Protein S29, Rps29) gene encodes a ribosomal protein that is a component of the 40S subunit. Also, RPS29 protein a member of the S14P family of ribosomal proteins and contains a C2-C2 zinc finger-like domain that can bind to zinc. Here, we performed evolutionary characterization of alternative splicing events in RPS29 gene via genomic PCR, RT-PCR amplification, and sequencing analysis. Interestingly, in RPS29 gene, two different alternative transcripts are identified by two different lineage-specific integration events of Alus (AluSg in apes and Alusx3 in Old World monkeys). We validated the

existence of the alternative transcripts by RT-PCR and sequencing analysis using primate samples. We conducted PCR amplification and sequencing procedures of lineage-specific AluSg and AluSx3 elements to investigate the integration time during the primate radiation. As a result, our in silico and experimental analysis shows that AluSg elements were integrated into our common ancestor genome after radiation events between apes and Old world monkeys. And AluSx3 elements integrated into the Old world monkey lineage only. We found that a novel transcript was created by exonization due to different Alu at the same locus in RPS29 gene.



Mapping the shape of the helmet onto the genomic phylogeny reveals that this trait is phylogenetically conserved across the tribes of treehoppers (Hemiptera: Membracidae).

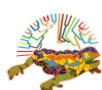
De la Mora Curiel M¹

¹Unam/enes Juriquilla

POSTER SESSION 2 - P02.225, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

We sampled treehoppers from the Mesoamerican region, extracting DNA to amplify approximately 2,500 ultraconserved elements (UCEs) and reconstruct the phylogenetic relationships within the group. Using geometric morphometrics, we analyzed the shape of the helmet structure in treehoppers and mapped it onto the phylogenetic tree to assess phylogenetic conservatism of similar forms. Our evolutionary question focused on whether

the complexity of this structure evolved multiple times independently or if a common bauplan precedes certain morphological forms. We successfully reconstructed a well-supported phylogeny for nearly all clades and found evidence of phylogenetic signal for the evolution of the helmet at the tribal level. This suggests that evolutionary innovations in this group emerge at the tribe level, from which species-specific forms are derived.



Milk transcriptomes provide insights into the evolution of pinniped lactation traits

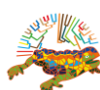
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POSTER SESSION 2 - **P02.238**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Exploring patterns of gene expression is an important complement to genomic selection scans for coding substitutions to help understand the genomic basis of adaptation. Pinnipeds (seals, sea lions, fur seals, and walruses) exhibit a range of breeding and lactation strategies, each requiring specialized adaptations for energy storage and efficient energy transfer to their offspring through high-fat, low-sugar milks. This diversity has evolved as pinniped species adapted to various latitudes and habitats, facing distinct selective pressures due to their unique need to separate land and sea for breeding and foraging. Although the physiological characteristics of these breeding strategies are relatively well understood, little is known about the genomic basis of variation in traits across species. We used RNAseq analysis to characterize patterns of gene expression in grey seal milk and blubber during early and late lactation to reveal insights into the mechanisms of energy storage and transfer. We collected milk, blood, and blubber samples from 10 mother-pup pairs on the Isle of May, UK, during early (day 6) and late lactation (day 16), November-December 2023. Using the DESeq2 pipeline, we analyzed how gene expression in these tissues change as lactation progresses, finding patterns consistent with known changes in metabolite profiles in milk and

blubber across lactation for lipids, carbohydrates, vitamins, and micronutrients. Comparison with milk fat transcriptome data from other mammals help identify key metabolic pathways and transcripts that are expressed in consistent ways across diverse mammals. Network analysis shows patterns of co-expressed genes that contribute to the rapid transfer of energy from mother seal to pup. Seal milk fat transcripts for proteins expressed in milk fat globule membranes were found at high abundance compared to other mammals, suggesting a possible mechanism contributing to the high fat content and stability of pinniped milks. The gene LALBA, which mediates lactose secretion in mammal milk, has previously been found to be absent in otariid (sea lion/fur seal) milk, but LALBA transcripts were expressed in grey seal milk at levels comparable to other mammals. This suggests that the reduced sugar content of pinniped milks is controlled by different mechanisms in otariids and phocids ("true" seals). Our findings uncover a comprehensive set of genes associated with high milk fat content and lipid mobilization and metabolism in blubber, providing the most detailed insight yet into this extraordinary evolutionary adaptation and offer insights into the diversity of lactation traits across mammals.



Modeling Sex-biased Gene Expression Evolution Across Firefly Phylogeny

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POSTER SESSION 2 - P02.240, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Fireflies (Lampyridae) exhibit various levels of sexual dimorphism, with extreme cases occurring repeatedly across the whole phylogeny. The sexual dimorphism is often linked to sex-biased gene expression, yet the evolutionary processes driving the divergence of sex-biased gene expression remain poorly understood. Phylogenetic comparative methods have been extended to analyze transcriptome data in a way that accounts for shared ancestry and enables inference of the evolutionary forces acting on gene expression. Among these methods, the stochastic Ornstein-Uhlenbeck (OU) process is widely used to model gene expression evolution under a phylogenetic framework. In most studies, only the mean expression values of each species are used as continuous traits, while within-species variances are either ignored or treated as nuisance parameters. However, relying solely on mean values can overlook important information about the adaptive process experienced by certain species, which may be captured by within-species variances. For example, small variances could be a signal of strong selection on the expression of a gene.

Materials and methods: In this project, we generated gene expression data from two body parts (heads and bodies) and multiple individuals of both sexes per firefly species. For model development, we extended the OU process to model mean expression levels

and within-species variances jointly under a Bayesian framework using RevBayes. Expression evolution for each sex and each body part is inferred independently. Then we contrast the patterns between sexes and body parts to understand the evolution of sex-biased gene expression across firefly phylogeny.

Results Preliminary results using the extended OU model revealed evidence of evolution of sex-biased gene expression in the body, while expression evolution in the head appears to be more unbiased across most genes and species. We observed a very weak correlation in gene expression variances between closely related species, suggesting that variance in gene expression changes rapidly across the whole firefly phylogeny.

Conclusion: Our approach treats within-species variances as a reflection of the adaptive processes shaping gene expression and thus includes the variances into the evolutionary modeling framework. By extending the OU process to jointly consider species means and within-species variances, we gain a more accurate and detailed understanding of how sex-biased gene expression evolves in Fireflies.

Keywords: fireflies, gene expression, Ornstein-Uhlenbeck process, within-species variances, RevBayes



Multimomics reveal associations between CpG methylation, histone modifications and transcription in a species that has lost DNMT3, the Colorado potato beetle

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POSTER SESSION 2 - P02.224, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Insects display exceptional phenotypic plasticity, which can be mediated by epigenetic modifications, including CpG methylation and histone modifications. In vertebrates, both are interlinked and CpG methylation is associated with gene repression. However, little is known about these regulatory systems in invertebrates, where CpG methylation is mainly restricted to gene bodies of transcriptionally active genes. A widely conserved mechanism involves the co-transcriptional deposition of H3K36 trimethylation and the targeted methylation of unmethylated CpGs by the de novo DNA methyltransferase DNMT3. However, DNMT3 has been lost multiple times in invertebrate lineages raising the question of how the links between CpG methylation, histone modifications and gene expression are affected by its loss. Here, we

report the epigenetic landscape of *Leptinotarsa decemlineata*, a beetle species that has lost DNMT3 but retained CpG methylation. We combine RNA-seq, enzymatic methyl-seq and CUT&Tag to study gene expression, CpG methylation and patterns of H3K36me3 and H3K27ac histone modifications on a genome-wide scale. Despite the loss of DNMT3, H3K36me3 mirrors CpG methylation patterns. Together, they give rise to signature profiles for expressed and not expressed genes. H3K27ac patterns show a prominent peak at the transcription start site that is predictive of expressed genes irrespective of their methylation status. Our study provides new insights into the evolutionary flexibility of epigenetic modification systems that urge caution when generalizing across species.



Multiple Origins, Singular Success: Genomic Evidence for Recurrent Hybridization in *Chenopodium album* s. str.

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POSTER SESSION 2 - **P02.235**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Polyploidy, particularly allopolyploidy resulting from interspecific hybridization followed by chromosome duplication, plays a key role in plant diversification and ecological success. *Chenopodium album* s. str. is one of the world's most widespread and morphologically variable weeds, yet the timing and mechanism of its origin as an allohexaploid ($2n=6x=54$) remained unresolved. Using RADseq data, this study investigated the evolutionary origin of *C. album* s. str. by analyzing its relationship to diploid progenitors with the 'BB' genome (*C. ficifolium*, *C. suecicum*, *C. ucrainicum*) and tetraploid species with the 'CCDD' subgenomic combination (*C. betaceum*, *C. glaucophyllum*, *C. novopokrovskyanum*, *C. striatiforme*). The genomic sequences were mapped to the chromosome-scale reference genome of *C. formosanum*, a closely related allohexaploid.

Results reveal that *C. album* s. str. does not genetically align with contemporary diploid

or tetraploid progenitors, suggesting its origin from now-divergent or extinct ancestors. Both its 'BB' and 'CCDD' subgenomes show higher genetic diversity than extant parental species, implying conservation of ancestral variation. Furthermore, 16 distinct sub genomic combinations were identified within *C. album* s. str., confirming its polytopic and repeated origin across geographic regions rather than ongoing hybridization. This explains the remarkable morphological and ecological plasticity observed across its range.

This research underscores *C. album* s. str. as an ancient, stabilized, and globally invasive polyploid, shaped by multiple hybridization events and fixed heterozygosity, offering parallels with other major polyploid crops such as *Triticum aestivum* (bread wheat). Understanding these patterns deepens our grasp of polyploid evolution, speciation, and weed adaptation on a global scale.



Multivalent Mysteries: How Termite Chromosome Chains Shape Evolution

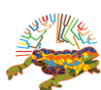
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POSTER SESSION 2 - P02.242, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Termites challenge long-standing assumptions about insect social evolution. One important difference compared to the social ants, bees and wasps lies in their chromosomes: termites are diploid, with X/Y sex differentiation observed in most species and males playing active roles across all castes and life stages. Adding to their uniqueness, termites exhibit multivalent chromosome chains during male meiosis—an intriguing and seldom seen feature of eukaryote evolution. These chains, in addition to other chromosomal formations, such as bivalent rings and rods, may serve to suppress recombination, thereby mitigating against the potentially harmful effects of inbreeding. Again, this positions termites as an important contrasting study system to other social insect groups, where recombination rates tend to be elevated. Multivalent chromosome chains may also play a significant role in speciation

processes by increasing the likelihood of chromosomal translocations and promoting genomic islands of divergence. In European *Reticulitermes*, variability in chain length and stability both between and within species provides a compelling model system for studying the evolutionary importance of multivalent chromosome chains at intra- and interspecific levels and at different evolutionary time scales. To investigate these dynamics, we collected over 200 colonies of *Reticulitermes* species across the Mediterranean, generating de novo reference genomes, comprehensive population genomic as well as karyotypic data from three recently diverged species. Our findings shed light on the intricate relationship between chromosomal architecture and evolutionary mechanisms in termites, offering new insight into how genome structure shapes species evolution.



Parallel Ranges, Divergent Histories: A Comparative Genomic Study of Two Brassicaceae Species

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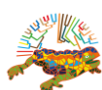
POSTER SESSION 2 - P02.228, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Studying the dynamics of populations and their genetic divergence with historical ecological and climatic events enhances our understanding of the mechanisms of evolution. Comparing the population dynamics and distribution of closely related species can illuminate the roles of common versus divergent ecological parameters in shaping trait and sequence diversity patterns and identify convergent genetic responses to global ecological changes. *Cardamine hirsuta* (*Cardamine*), a small crucifer related to *Arabidopsis thaliana* (*Arabidopsis*), exhibits both shared and unique traits, establishing it as a powerful complementary model for experimental research. The extent of range overlap and asymmetry between these two related species provides a useful framework for examining the influence of geography and ecology on speciation and adaptation. Our goal in this study was to assess whether comparable ecological and paleoclimatic drivers played a role in shaping the ancient distribution and migration patterns of the two species.

To investigate the comparative demographic histories of *Cardamine* and *Arabidopsis*, we re-sequenced 842 *Cardamine* accessions, primarily collected from Europe and Africa. We reconstructed the demographic history of *Cardamine* using classic site frequency spectrum (SFS) based and contemporary ancestral recombination graph (ARG) based methodology. In addition, we performed long-read sequencing on 75 *Cardamine* accessions to characterise the transposable element landscape across populations. We also constructed a *Cardamine* pan-genome graph to identify structural variants, enabling us to

compare population structure based on both small and large variants and to explore the contribution of structural variation to pericentromeric diversity.

Population genetic analyses of Pan-European *Cardamine* samples revealed a highly differentiated genetic group in the Iberian Peninsula, occupying a broader geographic range than its counterpart in *Arabidopsis* suggesting a greater ability to establish beyond glacial refugia. The Iberian and Balkan populations of *Cardamine* were found to be deeply diverged, in contrast to the post-glacial expansion pattern observed in European populations of *Arabidopsis*. African genomes of *Cardamine* revealed two populations: North African and East African. The East African population of *Cardamine* exhibited high genetic divergence, with no comparably divergent population detected in *Arabidopsis* across Europe or Africa. Additionally, the North African *Cardamine* population showed a complex history of lineage sharing with both Iberian and Balkan groups—a pattern not observed in *Arabidopsis*. We also identified both shared and distinct patterns of population structure among *Cardamine* populations, based on short variants, structural variants, and transposable elements. In this study, we uncovered strikingly contrasting demographic histories between two closely related species within the Brassicaceae family. Our findings highlight the complex interplay of historical processes, geographic factors, and ecological dynamics in shaping population histories and genetic divergence in these species.



Recently evolved, stage-specific genes are enriched at life stage transitions in flies

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POSTER SESSION 2 - **P02.258**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Understanding how genomic information is selectively utilized across different life stages is essential for deciphering the developmental and evolutionary strategies of metazoans. In holometabolous insects, the dynamic expression of genes enables distinct functional adaptations at embryonic, larval, pupal and adult stages, likely contributing to their evolutionary success. While *Drosophila melanogaster* has been extensively studied, less is known about the evolutionary dynamics that could govern stage-specific gene expression. To address this question, we compared the distribution of stage-specific genes, i.e., genes expressed in temporally restricted developmental stages, across development of *D.melanogaster* and *Aedes aegypti*. Using tau-scoring, a computational method to determine gene expression specificity, we found that, on average, a large proportion of genes (20 to 30% of all protein-coding genes) in both species exhibit restricted expression

to specific developmental stages. Phylostratigraphy analysis, a method to date the age of genes, further revealed that stage-specific genes fall into two major categories: highly conserved and recently evolved. Notably, many of the recently evolved and stage-specific genes identified in *A.aegypti* and *D.melanogaster* are restricted to Diptera order (20 to 35% of all stage-specific genes), highlighting ongoing evolutionary processes that continue to shape life-stage transitions. Overall, our findings underscore the complex interplay between gene evolutionary age, expression specificity, and morphological transformations in development. These results suggest that the attraction of genes to critical life stage transitions is an ongoing process that may not be constant across evolutionary time or uniform between different lineages, offering new insights into the adaptability and diversification of dipteran genomes.



Recurrent genomic dynamics linked to parallel evolution of secondary phytophagy in Hymenoptera

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POSTER SESSION 2 - **P02.245**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The phytophagous lifestyle is a key innovation in insects and has evolved in only one third of all insect orders. The evolution of phytophagy involves fundamental behavioural and morphological changes accompanied by chemosensory and metabolic adaptations. To date, the genomic basis and genetic innovations related to evolutionary dietary shifts are poorly understood. Here we focus on two monophyletic groups within the Hymenoptera, Aculeata and Chalcidoidea, that secondarily developed larval phytophagy independently several times. This phytophagy can be characterized by the feeding of pollen, nectar, plant oil or in the formation of plant galls. To shed light on evolutionary processes that shaped the diversity of nutritional adaptations in Hymenoptera we address the

following main research questions: (1) Is parallel evolution at the phenotypic level reflected by parallel genome evolution? And (2) did similar genomic innovations appear when independent lineages realized convergent dietary transitions? Here I want to introduce the current state of the project and give insights into newly sequenced genomes of representative phytophagous aculeates. Especially, gene family size evolution of potentially important gene families involved in chemoperception and detoxification will be compared between different phytophagous species. Additionally the situation in closely related zoophagous species will be shown. This information will be helpful to assess the role of gene family expansion and gene loss in context of the evolution of secondary larval phytophagy in Hymenoptera.



Sex-biased gene expression evolution in extreme sexually dimorphic fireflies

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POSTER SESSION 2 - **P02.257**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Sexual dimorphism varies widely among species, often arising from differential selection pressures on males and females. Sex-biased gene (SBG) expression is a key mechanism for resolving sexual conflict, yet the evolutionary forces shaping SBG expression remain unclear for most species fireflies (Lampyridae), with an evolutionary history ~150 million years, display a remarkable diversity in sexual dimorphism, with species ranging from an unnoticeable sexual dimorphism to females retaining neotenic/immature traits, making them an ideal system to study the evolution of SBG expression. Yet, only recently, sexual dimorphism in fireflies has started to be studied.

Fireflies provide a particularly compelling system to study evolutionary dynamics and evolutionary forces due to their repeated evolution of extreme sexual dimorphism across diverse lineages. Likewise, their unique life history traits, including variation in female flight ability and bioluminescent signaling, make them an ideal model for understanding how selection (e.g., stabilizing, directional) and genetic drift interact to shape SBG expression.

The project aims to investigate how stabilizing selection, directional selection, and genetic drift influence the evolution of SBG expression across multiple firefly species under a recently phylogenetic reconstruction consolidated in our research group. This firefly phylogenetic reconstruction sets the backbone for further phylogenetic comparative analyses across the firefly species and resolves the unknown firefly phylogenetic relationships. For this, we obtained RNA-seq of 20 firefly species

with different sexual dimorphism, distributed globally. Initially, we generated new firefly reference genomes that were annotated and finally aligned with STAR. Differential gene expression was computed in R with DEseq2, and SGB were depicted with p-adjusted value < 0.05 and an absolute log2 fold change (LFC) > 1.5. Phylogenetic comparative methods in SBG expression were conducted in R.

Overall, we found more differentially expressed genes in heads than in fireflies' bodies. Also, a large number of male-biased genes (MBGs) compared to female-biased genes (FBGs) in the bodies, with an opposite trend in heads (i.e., more FBGs than MBGs). Similarly, across the phylogeny, the subfamily Lamprohizinae showed more SBGs in heads than any other subfamily. These patterns suggest tissue-specific and lineage-specific evolution of SBGs, potentially driven by divergent selective pressures on sexually dimorphic traits such as bioluminescence and flight.

By integrating comparative genomics and SBG expression in fireflies, we show that SBG expression is lineage-specific, influenced by a combination of stabilizing, directional selection, genetic drift and life history traits of each firefly species. These findings deepen our understanding of how sex-specific selection and regulatory evolution contribute to the emergence of morphological and behavioral dimorphism, with broader implications for evolutionary biology. Ongoing sampling will expand our taxonomic coverage and further refine these evolutionary inferences.



Stingless and Superorganismal: Multi-omic analysis reveals population differentiation and signatures of social evolution in *Tetragonula* bees

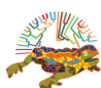
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POSTER SESSION 2 - **P02.226**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Stingless bees in the genus *Tetragonula* are social insects with a fully sterile worker caste, and are therefore well-placed to provide insights into the genomic changes associated with ‘superorganismal’ life histories. Here we assemble the genome of *Tetragonula carbonaria* and characterize the population structure and divergence of both *T. carbonaria* and its cryptic congener *T. hockingsi* in eastern Australia, revealing three distinct populations for *T. carbonaria* and two partially-differentiated subpopulations for *T. hockingsi*. We then combine our genomic results with RNA-seq data from different *T. carbonaria* castes (queens, males, workers) to test two hypotheses about genomic adaptations in social insects: the ‘Relaxed Constraint’ hypothesis, which predicts indirect, and therefore relaxed, selection on worker-biased genes; and the ‘Adapted Worker’ hypothesis, which predicts intensified

positive selection on worker genes due to their evolutionarily novel functions. Although we do not find a direct signal of either weaker purifying selection or elevated positive selection in worker-biased genes based on deviations from neutral expectations of nucleotide change between the two species, other evidence does support a model of relaxed selection on worker-biased genes: such genes show higher nucleotide diversity and greater interspecies divergence than queen-biased genes. We also find that differentially caste-biased genes exhibit distinct patterns of length, GC content and evolutionary origin. These findings, which converge with patterns found in other social insects, support the hypothesis that social evolution produces distinct signatures in the genome. Overall, *Tetragonula* bees emerge as a valuable model for studying the genomic basis of social complexity in insects.



Support for the ovarian ground plan hypothesis in the evolution of eusociality from transcriptome analyses of halictid bees

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POSTER SESSION 2 - **P02.233**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Eusociality represents a significant evolutionary innovation, having evolved multiple times across insect lineages, including four times independently in the bees. The defining feature of eusociality in insects is reproductive division of labour, i.e. queen and worker castes; queens are responsible for reproduction while workers are responsible for brood care, foraging (resource acquisition), and nest protection. One prominent mechanistic theory to explain how eusociality might have arisen from a solitary ancestor is the ovarian ground plan hypothesis (OGPH), which suggests that maternal traits in solitary ancestors, encompassing both brood care and reproduction, were subsequently decoupled in social descendants where the two tasks are undertaken separately by queens (reproduction) and workers (brood care). Here we aimed to investigate the molecular implications of the OGPH in one lineage of sweat bees (Hymenoptera: Halictini), where eusociality has arisen once, been further elaborated, and subsequently lost multiple times.

We generated transcriptome data of adult female bees, including intraspecific

comparison (across castes within social species) as well as interspecific comparison (across solitary and social species), focusing on brain and fat body tissues. Our results revealed a decoupling of transcriptomic profiles in alignment with the respective behavioural roles of females. In the intraspecific comparison of the eusocial *Lasioglossum marginatum*, queen transcriptomes were clearly differentiated from those of workers and foundresses. In interspecific comparison, transcriptomes of foundresses of *L. marginatum* overlapped with those of nesting females of the derived solitary *Halictus quadricinctus* and the ancestrally solitary *Dufourea dentiventris*, as predicted based on their functional equivalence. These results suggest that the different social phenotypes that are the hallmark of eusocial systems (queens and workers) have emerged from evolutionary changes in the regulation of expression of ancient genes, constitute evidence for the OGPH and moreover support the role of phenotypic plasticity in driving evolutionary innovation.



Testis Transcriptome Evolution in the Absence of Sperm Competition: Insights from Pregnant Males

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POSTER SESSION 2 - P02.231, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Male pregnancy in syngnathid fishes (pipefishes and seahorses) is a striking evolutionary innovation that leaves distinct signatures in the genome and transcriptome. In these species, male pregnancy eliminates sperm competition, providing a unique opportunity to investigate the role of sexual selection in shaping the evolution of male reproductive genes. Using comparative genomics and transcriptomics across syngnathids and other fishes with intense

sperm competition, we identify these molecular signatures. Syngnathid male reproductive genes evolve more slowly, show broader expression across tissues, and lack key protein functional categories related to fertilization. This work illustrates how the relaxation of sexual selection can reshape genome and transcriptome evolution, offering a compelling case study of how phenotypic novelty drives molecular change.



The Emergence of Diatom Heterotrophy on Brown Algal Polysaccharides through Horizontal Gene Transfer, Gene Duplication and Neofunctionalization

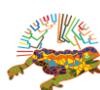
Lim Z¹

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POSTER SESSION 2 - P02.218, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

A major goal of evolutionary biology is to identify the genetic basis for the emergence of complex adaptive traits. Diatoms are ancestrally photosynthetic microalgae. However, a small group in the genus *Nitzschia* have become free-living secondary heterotrophs, completely losing their ability to photosynthesize. How these diatoms acquire energy and the genetic basis for their transition to obligate heterotrophy remains unclear. *Nitzschia sing1* is a non-photosynthetic diatom isolated from algal debris in the intertidal zone of Sentosa Island, Singapore. In this talk, I will present evidence for the genetic basis of its catabolism of the brown seaweed cell wall polysaccharide alginate. *N. sing1* obtained an endolytic alginate lyase enzyme through horizontal gene transfer (HGT) from a marine

bacterium. Subsequent gene duplication through unequal crossing over and transposition led to 91 genes in three distinct gene families. One family retains the ancestral function to cleave alginate polymers in an endolytic manner. By contrast, the two other families underwent domain duplication, gain, loss, rearrangement and mutation to encode novel functions accounting for oligosaccharide import through the endomembrane system and the exolytic production of alginate monosaccharides. Together, these results show how a single HGT event, followed by gene duplication and neofunctionalization, led to alginate catabolism and access to a new ecological niche.



The expanded *Bostrychia moritziana* genome unveils evolution in the most diverse and complex order of red algae

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POSTER SESSION 2 - P02.221, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Red algae form an ancient lineage of eukaryotes that were among the first to evolve multicellularity. Theory has it that the common red algal ancestor experienced a dramatic reduction in genomic content early in red algal evolution. Our comparative analysis of genome size variation across red algae suggests at least three independent origins of secondary genome size expansion, one of which is traced to the emergence of the Ceramiales, the largest and most diverse order of red algae. Despite their overwhelming diversity, we still lack comprehensive genome data from this highly-evolved group of red algae. Here, we report a chromosome-level genome assembly of *Bostrychia moritziana*, a prominent member of the Rhodomelaceae family of the Ceramiales. The *Bostrychia* genome is among the largest red algal genomes sequenced to date. Interestingly, this expansion is not a result of polyploidy or ancient whole genome duplications, but is rather driven by the dramatic proliferation of a single lineage of giant *Plavaka* DNA transposons. These elements account for a

third of the genome alone and have thus played a major role in shaping genome architecture. Consistent with increased genome size, we identify an increased gene content in *Bostrychia* shaped both by *de novo* gene emergence and by the amplification of gene families shared with other Ceramiales, providing key insight into the genetic adaptations underpinning the evolutionary success of this species-rich order. Furthermore, our sex-specific genome assemblies resolve the UV sex chromosomes in *Bostrychia*, which feature expanded gene-rich sex-linked regions. Notably, each sex chromosome harbours a TALE-HD transcription factor orthologous to ancient regulators of haploid-diploid transitions in other multicellular lineages. Overall, our work sheds light on the genomic innovations and adaptations underlying red algal diversity and highlights how this highly successful group of red seaweeds can provide insight into the evolutionary origins and universal principles underpinning complex multicellularity.



The genomic and developmental basis of an exaggerated sexual signal in the stalk-eyed fly *Teleopsis dalmanni*

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POSTER SESSION 2 - P02.227, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Sexual selection is a powerful evolutionary force, responsible for generating striking and exaggerated novel traits. In males, these traits can serve as honest signals of male quality, providing females with reliable cues to assess potential mates. However, the developmental processes and genes underlying the evolution of exaggerated male traits remain elusive in most species. Stalk-eyed flies are a specious group of insects that have evolved highly exaggerated eye-stalks, a novel evolutionary innovation in Dipterans that can vary substantially in length between sexes and species. Here, we use single-cell RNA sequencing and developmental imaging to test the developmental and genomic basis of eye-

stalk formation in *Teleopsis dalmanni*. First, we generated a single-cell atlas for the developing eye-antennal disc and perform comparative analysis with *Drosophila* to identify conserved and divergent transcriptomic features responsible for the evolution of this novel trait. Next, we harnessed fluorescent imaging to interrogate fine-scale spatiotemporal expression of key developmental genes, uncovering novel expression domains absent in *Drosophila* and assessing their potential roles in eye-stalk development. By integrating these approaches, we provide key insight into the genomic and developmental processes that drive sexual trait evolution and contribute to the emergence of evolutionary innovations.



The Genomic Basis of Convergent Fusiform Body Evolution in Lake Tanganyika Cichlid Fishes

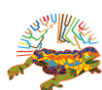
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POSTER SESSION 2 - **P02.247**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Locomotion-related traits are a major axis of morphological diversification in vertebrates. In the aquatic realm, the repeated evolution of streamlined, fusiform bodies in unrelated lineages is considered a non-trivial convergent adaptation that enhances swimming performance in open water. Among ray-finned fishes, which make up over half of all living vertebrate species, this body-plan has evolved multiple times and is often associated with transitions from benthic to pelagic habitats. However, despite the pervasiveness of this pattern across the Tree of Life, our understanding of the genetic basis of fusiform body-plan evolution is still elusive. Here, we present the first assessment of the macroevolutionary dynamics and molecular mechanisms underlying the convergent evolution of the fusiform body in one of the most outstanding examples of adaptive radiation—the cichlid fishes in Lake Tanganyika. To comprehensively characterize their post-cranial axial skeleton, we used X-ray imaging to analyze over 2,000 specimens, spanning the full diversity (233 species) of endemic tribes. The vertebral column of Tanganyika cichlids is divided into two distinct regions: the abdominal region, where vertebrae bear ribs, and the caudal region, characterized by vertebrae with fused haemal spines. We found that the number of vertebrae can increase independently in the abdominal and caudal regions of the vertebral column, but changes in vertebrae elongation are highly integrated in both regions. These findings align with broader patterns observed in actinopterygians, supporting the hypothesis that abdominal vertebral number, caudal vertebral number, and vertebral elongation are regulated by distinct

developmental modules. The Tanganyika cichlid radiation includes two primarily benthic lineages that have independently colonized the water column. Phylogenetic comparative analyses revealed that pelagic lineages converged toward a similar axial skeleton configuration. Notably, both lineages evolved streamlined, fusiform bodies, achieved primarily through episodic expansions of vertebrae counts in the abdominal region. To uncover the genetic basis of these changes, we used two genome-wide association (GWAS) methods: a general linear model (GLM) and a phylogenetic generalized least squares (pGLS) approach, which accounts for shared evolutionary history. Leveraging information from 1,066 genomes, we generated a variant call set and estimated allele frequencies across species. By intersecting SNPs identified by both methods, we detected clusters of strongly associated variants shared among lineages exhibiting episodic expansions in vertebral counts and elongation. Remarkably, variants linked to abdominal count, caudal count, and elongation showed little overlap, reinforcing the idea of modular genetic control. While some variants were linked to known patterning genes such as *hoxda*, we also found an unexpected enrichment of genes involved in skeletal, neuronal, and muscular development across all traits. By integrating dynamics of pulsed evolution with shared genetic variation, we demonstrate how the regulation of developmental pathways can drive rapid morphological changes, providing a molecular foundation for the episodic evolution of phenotypic traits that facilitate transitions into new adaptive zones along the water column gradient.



The genomic basis of Lepidoptera diversity and evolution

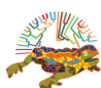
Mulhair P¹, Holland P²

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POSTER SESSION 2 - **P02.223**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Lepidoptera (moths and butterflies) represent one of the most diverse animal orders with over 160,000 described species. The diversity in form and behaviour within this order can be attributed to adaptations at the genetic level. Studies of gene content and genome evolution in Lepidoptera have been limited to certain well-studied species, owing to the lack of high-quality genome data for most families. Recent construction of a large number of chromosome-level assemblies provides the opportunity to analyse the role of gene and genome evolution in the diversification of this order of insects. Here, we provide a genome-wide analysis of protein coding gene evolution across Lepidoptera. We assess order-wide rates of evolution, patterns of gene gain, duplication and loss, and conservation of gene microsynteny across 497 arthropod species, including 454 lepidopteran species. To analyse these genomic features, we first constructed a species tree using 989 BUSCO genes shared across 90% of the taxa in our dataset. Rates of sequence evolution across the tree were assessed by measuring dN/dS across the supermatrix of 989 aligned genes. We find the highest shifts in rates of evolution in the deep nodes of the lepidopteran tree, in particular coinciding with periods of rapid diversification in lepidopteran evolution, such as after the emergence of Ditrysia. Comparing rates of evolution between lepidopteran families reveals variable patterns of genetic diversity, with the families Pieridae, Lycaenidae, and Geometridae displaying the highest overall genome-wide evolutionary rates. Assessing

gene turnover reveals dramatic bursts of gene gain in the early branches of the Lepidoptera phylogeny. In particular, the two nodes subsequent to the emergence of Ditrysia (655 and 1,331 genes gained) and the node coinciding with the emergence of Apoditrysia (2,356 genes gained) show the highest number of gene gain events. Despite high and unequal rates of gene loss closer to the tips of the tree, this increase in gene number in the early evolution of Lepidoptera means that these species differ significantly in their gene content when compared to other insect orders. Finally, we availed of the high-quality genomic data to analyse the conservation of local gene synteny across Lepidoptera. Similar to genome macrosynteny, we find a high and consistent rate of conserved microsynteny across the order. We also uncover family specific gene clusters, for example in Lycaenidae (568 gene clusters), Tortricidae (222 clusters), and Geometridae (119 clusters). One example of family specific gene order revealed extreme copy number variation and gene translocation within the Lipocalin gene superfamily in Lycaenidae. Specifically, we found high rates of duplication of the bilin-binding proteins within all lycaenid species, with copy number ranging from 11-24 copies (mean 1.5 gene copies outside of Lycaenidae). The bilin-binding proteins are involved in determination of blue pigmentation and wing colouration and this finding points to a link between gene duplication and the blue colouration in the bodies of many lycaenid butterflies.



The transcriptomic basis of phenotypic diversity in stick insects

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POSTER SESSION 2 - **P02.243**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Identifying the role that different regulatory processes play in the development and maintenance of phenotypic variation is essential for understanding the molecular routes to adaptation. An ideal way for studying the molecular basis of trait diversity is to compare species with different levels of phenotype complexity, such as species with separate sexes and parthenogenetic species. In sexually reproducing species, males and females often have conflicting phenotypic optima and experience different selection pressures on many shared traits. By contrast, because parthenogenetic species consist solely of females, only the female phenotypes are subject to selection and they are thus free to evolve without potential constraints imposed by simultaneous selection on male phenotypes. We leverage the multiple,

independent transitions from sexual to parthenogenetic reproduction that have occurred in the *Timema* stick insect genus to investigate the evolution of different regulatory mechanisms under different levels of phenotype complexity. We use both short-read and PacBio long-read RNA-sequencing from multiple somatic and reproductive tissues in a comparative analysis of replicate sexual and parthenogenetic species pairs to identify convergent changes in gene expression, alternative splicing, and gene co-expression networks. We also test the complementarity of these molecular mechanisms in shaping phenotypic diversification and assess how the genomic architecture shifts following the loss of sex and loss of genetic conflicts.



“Tinkering loci” rapidly generate new genes in *Drosophila*

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POSTER SESSION 2 - **P02.261**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Where do new genes come from? Reconstructing the evolutionary history of a novel *Drosophila* gene has revealed what I propose to call “tinkering loci”: genomic regions into which genes are copied, rearranged, and, surprisingly often, transcribed. Common rearrangements at these loci include the creation of gene fragments (standalone pieces of ancestral genes), reassorted genes (genes made from pieces of the same ancestral gene, but duplicated and/or rearranged), and chimeric genes (genes made from pieces of multiple ancestral genes); in many cases, reassorted genes and chimeric genes are made from three or more distinct pieces. The same loci have birthed wholly different new genes in

different members of very young (<10 My) lineages, suggesting that they have specialized properties making them well-suited to this task. While there is evidence for a modest number of tinkering loci in many lineages, they are extremely abundant in only a few. I will provide a phenomenological overview of these loci and how they were found, provide an estimate of their rate of gene birth, propose a simple mechanistic model for how they work and why they exist, and speculate on what I see as the implications of the existence of evolutionarily rare loci specialized for generating new genes from pieces of old ones for our understanding of animal gene evolution.



Transposable elements contribute to *Anopheles coluzzii* and *An. gambiae* adaptation to urban environments

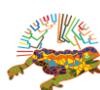
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POSTER SESSION 2 - **P02.259**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Anopheles gambiae and *An. coluzzii* mosquitoes are major human malaria vectors in Africa, accounting for most of the disease transmission. While urban environments were until recently considered to be unfit for *Anopheles* larvae development, these mosquito species have rapidly adapted to polluted habitats in the last decades, posing challenges for malaria control. Therefore, understanding the genetic mechanisms underlying this adaptability is critical to advancing malaria eradication efforts. In this study, we generated 199 whole-genome sequences from urban *An. coluzzii* and *An. gambiae* individuals collected across the four major cities of Central Africa, and incorporated an additional 176 genomes from rural areas obtained from existing datasets, covering a total of six countries. Taking advantage of recent high-quality long-read assemblies for both species, our analysis focused on identifying both SNPs and transposable elements (TEs) as genetic variants potentially involved in urban adaptation. We generated the first manually curated TE library for *An. gambiae*, comprising 295 consensus sequences, including 53 newly identified TE families. By combining three TE annotation tools we identified 5,462 and

4,773 euchromatic TE insertions present at high population frequencies (>10%) in *An. coluzzii* and *An. gambiae* populations, respectively, which may contribute to adaptive processes. By applying genome scans capturing adaptation at different timescales, including population differentiation and genotype-environment association analyses, we identified both known and novel candidate genes associated with urban adaptation in both species. Notably, half of these genes are associated with TE insertions present at high population frequencies. In *An. coluzzii*, most of the genes identified in response to urban adaptation were involved in the metabolism of toxic compounds, as for example the cluster of glutathione S-transferases gene family, and were detected through both recent and old selective signals. In contrast, in *An. gambiae*, candidate genes were not enriched for any biological process. Finally, we found that approximately 40% of the genes showing signatures of selection in both species likely result from introgression, many of them also associated with TEs. Altogether, our findings highlight the potential role of TEs, alongside SNPs, as drivers of urban adaptation in *Anopheles* mosquitoes.



Waddington revisited: Standing genetic variation drives the evolution of acquired traits

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POSTER SESSION 2 - P02.239, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Genetic assimilation, first described by Waddington, is the evolutionary process by which environmentally induced traits become genetically encoded. Despite its historical significance, the molecular basis of this process remains largely unexplored. In our study, we revisit Waddington's classic heat stress experiments using the ectopic wing vein (EV) phenotype in *Drosophila melanogaster*. We combined genomic, transcriptomic, and epigenomic analyses in both outbred and inbred natural populations to disentangle the contributions of induced versus pre-existing variation to trait evolution. Our findings show that the evolution of the EV trait is driven predominantly by selection on standing genetic variation, rather than by novel mutations or environmentally induced stable epigenetic changes. We identified a

transposable element insertion in the 3' untranslated region of the *Cad96Ca* gene, associated with reduced gene expression and the EV phenotype. This polymorphism persists in inbred populations due to a large chromosomal inversion that limits recombination and preserves haplotype structure. These results suggest that trait assimilation under environmental stress may arise via selection on cryptic alleles already present in the population, rather than through de novo genetic or stable epigenetic mutations. Our work sheds light on the genetic architecture of evolutionary innovations and highlights the roles of regulatory variation, mobile genetic elements, and chromosomal inversions in shaping adaptive phenotypes.



Deconstructing the contribution of lineage-specific transposable element insertions to nervous and sensory system diversification in Lake Malawi cichlid fishes

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MEETING ROOM 113, August 19, 2025, 2:00 PM - 5:00 PM

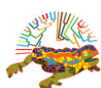
Diverse and complex brains are firmly associated with the evolutionary success of vertebrates, enabling processing of environmental stimuli and formulation of behavioural strategies optimising fitness. Among them, fishes display striking interspecific variation in brains and sensory systems often linked to habitat use, feeding strategies and social behaviours. However, the molecular mechanisms underpinning this adaptive diversity remain poorly understood. Here, we leverage the eco-morphological diversity of African Rift Valley's Lake Malawi cichlid radiation to explore how differences in early neural development and gene regulation contribute to adult brain variation across species within this teleost clade.

Using μ CT scans, we identified significant volumetric differences in adult forebrain regions, including telencephalon and diencephalon, between two ecologically divergent cichlid species: the generalist *Astatotilapia calliptera* and the pelagic predator *Rhamphochromis* sp. 'chilingali'. These neuroanatomical differences are mirrored by divergence in expression levels of key neural patterning genes (e.g. *shh*, *wnt1*, *otx2a*, *fgf8a*) during early embryonic brain morphogenesis. Among these, *fgf8a* emerged as a strong candidate gene due to its central role in vertebrate brain development and previously identified signatures of positive selection within its locus in Malawi cichlids. Using in situ Hybridisation Chain Reaction (HCR), we uncovered inter-specific differences in brain *fgf8a* expression domains across embryogenesis, whereas CRISPR/Cas9-mediated disruption of *fgf8a* revealed marked defects in brain,

craniofacial structures, and body axis formation in cichlid embryos. Taken together, our findings implicated divergence in *fgf8a* regulation as a possible mechanism of nervous system diversification between cichlid species.

We combined functional, transcriptomic, and comparative genomic approaches to dissect the regulatory architecture surrounding *fgf8a*. Strikingly, we uncovered lineage-specific structural variation in the Malawi cichlid *fgf8a* locus, including multiple transposable element (TE) insertions proximal to the gene. Motif analysis revealed that several of these elements harbour binding sites for transcription factors (TFs) associated with neural and sensory development, as well as pigmentation. Importantly, we identified small deletions in *Rhamphochromis*, resulting in a predicted loss of TF binding sites. Using transgenic zebrafish reporter assays, we confirmed the intrinsic and species-specific regulatory potential of these cichlid-specific TEs, driving reporter expression in multiple tissues during embryogenesis. These comprised several brain regions, including hindbrain and *fgf8a*+ pineal gland, as well as lateral line, eye, skin, muscle and heart. The broad activity in the interspecific transgenic assay highlights the wide regulatory potential of these elements, possibly extending beyond modulation of neighbouring *fgf8a* in their endogenous context.

Ongoing work is expanding this framework to dissect the regulatory activity and function of these elements in cichlids and test the association between sequence variation in these novel regulatory elements and neural and sensory diversity across the Lake Malawi cichlid radiation.



Evolution of salivary protein repertoire in true bugs and its role in dietary adaptations

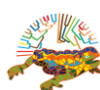
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¹Justus Liebig University Giessen, ²University of Toulouse, ³Max-Planck-Institute for Chemical Ecology

MEETING ROOM 113, August 19, 2025, 2:00 PM - 5:00 PM

True bugs (Heteroptera) form a suborder of more than 40.000 species within the insect order Hemiptera. Unlike other exclusively phytophagous hemipteran suborders, the Heteroptera have evolved a range of dietary habits, including zoophagy, phytophagy, hematophagy, mycophagy and mixed diets such as zoophytophagy. True bugs have piercing-sucking mouthparts and their saliva plays an important nutritional role, as it is used for extraoral digestion and, in zoophagous species, also for envenomation. However, the evolution of the salivary protein repertoire and its role in dietary adaptations of true bugs is unknown. This project aims at investigating the molecular and genetic basis responsible for dietary shifts and adaptations in the Heteroptera. We have collected genomic data of more than 50 heteropteran species covering most families and diets, and applied unsupervised phylogenomic comparative approaches to identify gene family expansions and contractions associated with different diets.

Most differences were found between zoophagous (venomous) and non-zoophagous species. Using additional tissue-specific RNAseq data, we identified several gene families encoding for salivary proteins that are significantly expanded in zoophagous species compared to non-zoophagous species. These include putative venom-related proteins such as protease inhibitors, venom allergens and uncharacterized venom protein families. Using heterologous gene expression and bioactivity assays for toxicity, antimicrobial and anticoagulant activity, we aim to functionally characterize candidate proteins to understand their role in dietary adaptations and venom evolution in true bugs. Our study will shed new light on the molecular basis of adaptive evolution in insects and will help to understand the remarkable dietary diversification in Heteroptera.



Evolutionary novelties escape loss by recruiting life-history trade-offs

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MEETING ROOM 113, August 19, 2025, 2:00 PM - 5:00 PM

The evolutionary origin of novel traits is hotly debated. We investigated how beneficial variants escape loss due to drift and genetic constraints during the earliest stages of adaptation using “small-wing” crickets that evolved 8 years ago in Hawaii under pressure from eavesdropping parasitoids. Small-wing males have genetically miniaturized wings that produce novel songs, protecting them from parasitoid attack but interfering with mate attraction. Combining bioacoustics, functional and comparative genomics, competitive fitness assays and genetic simulations, we find that genetic recruitment of a common life-history trade-off between

reproduction and flight promoted this rapid evolution. Crickets in this small island population do not fly, so both sexes gain life-history benefits from wing reduction without paying the typical dispersal costs. These indirect benefits allowed small-wing genotypes to escape loss by counterbalancing genetic constraints and genetic drift. Positive pleiotropy via genetic co-option of life-history trade-offs may be an underappreciated mechanism powering adaptive evolution and evolutionary innovation in nature.



Exploring the Emergence of Newly-Evolved Expressed Open Reading Frames (neORFs) in *Drosophila*

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MEETING ROOM 113, August 19, 2025, 11:00 AM - 1:00 PM

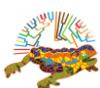
Background: Genes can arise not only through the modification of existing genes but also entirely from scratch. For this to occur, these de novo genes need to gain the necessary mutations to be transcribed, as well as acquire an open reading frame (ORF) that enables translation into a protein. The exact mechanisms that allow de novo genes to emerge from noncoding regions of the genome are not fully understood. This is also true for the frequency at which they emerge, spread within a population, and eventually achieve functionality. Furthermore, studying de novo genes can be challenging due to inconsistencies between detection pipelines or quality differences between ingroup and outgroup genomes. These problems can lead to inaccurate estimates regarding the age or quantity of de novo genes.

Methods: Here, we aim to explore the dynamics of de novo gene emergence by sequencing the genomes and transcriptomes of multiple populations across six *Drosophila* species with divergence times of up to 40 million years. To overcome previous

challenges, we employ a consistent assembly and annotation strategy. Our focus lies especially on studying the early stages of de novo gene emergence. To do this, we made use of DESwoMAN, a recently developed pipeline designed to identify newly-evolved expressed ORFs (neORFs) from transcriptomes.

Results: We show that neORFs are predominantly specific to a single species, though a subset is shared across several of them. Moreover, we find that neORFs differ from protein-coding genes in several features such as length, GC content, and expression level. Finally, we identified their noncoding homologs in outgroup genomes which enabled us to examine the mutations allowing these neORFs to emerge.

Conclusion: In conclusion, neORFs exhibit distinct features compared to protein-coding genes. Additionally, the frequency of mutations in their noncoding homologs increases with phylogenetic distance, though not all of them are equally common. Taken together our results provide new insights into the early stages of de novo gene emergence.



From digestion to communication: molecular evolution during the rise of a social fluid

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MEETING ROOM 113, August 19, 2025, 2:00 PM - 5:00 PM

Cooperation begins with connection. In diverse ant lineages, the crop has been co-opted for trophallaxis—a mouth-to-mouth transfer of fluid that distributes food and endogenous proteins among colony members, forming a social circulatory system. Here, we examine the molecular and evolutionary transformation of the ant digestive fluid into a medium for collective physiology. By analyzing crop fluid proteomes across 28 species from 7 ant subfamilies and outgroups with variable use of social regurgitation, we identify hundreds of proteins that were recruited, conserved, or downregulated during the evolution of trophallaxis. Integrating these data with 178

ant genomes, we reveal recurrent molecular innovations, including gene duplications, positive selection, and regulatory shifts in protein abundance, that shaped the evolution of this social fluid. Our findings demonstrate how behavioral shifts can drive molecular evolution and repurpose physiological systems, illuminating the molecular evolution of a social fluid that mediates inter-individual coordination. This work provides a framework for understanding the evolution of socially transmitted materials and reveals fundamental principles underlying the molecular basis of cooperation and sharing systems across the tree of life.



Genomic determinants of gall formation and lifestyle transitions in Cynipoidea

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MEETING ROOM 113, August 19, 2025, 11:00 AM - 1:00 PM

The superfamily Cynipoidea (Hymenoptera) includes a diverse array of insects that have undergone multiple transitions between parasitoidism, gall induction, and inquilinism. Gall-inducing species manipulate their host plants to produce specific structures that protect and feed their larvae. In contrast, inquilines lay their eggs within galls induced by other species, most of the time replacing the inducer's progeny. Finally, parasitoids, a likely ancestral lifestyle within Hymenoptera, develop within other insect hosts, eventually killing them. These shifts between distinct ecological strategies have required substantial biological and physiological changes that must have left characteristic genomic signatures. In this study, we investigate the genomic bases of lifestyle transitions in Cynipoidea using a comparative phylogenomic framework focusing notably on convergent molecular adaptations, expansion and reduction gene family, and changes in selective pressure regimes. We analyze a dataset of more than 50 genome assemblies representing the diversity of lifestyles, namely parasitoid, gall-inducing, and inquiline species. These include both publicly available and newly sequenced genomes, complemented by transcriptomic data for gene annotation. After the construction of orthogroups, we are tracking gene family dynamics (expansion and contraction), as well as shifts in selective pressure regimes in orthologous

genes, putatively correlating with the transition to a new lifestyle. Special attention is given to genes involved in ovarian follicle cell development—previously found important for gall induction in a recent transition toward gall inducer—and to families of genes coding for plant cell wall-degrading enzymes (PCWDEs), horizontally acquired from bacteria—that were found as involved in gall induction and/or development in Cynipidae lineage-specific studies and, more generally, in evolution of herbivory in insects. We hypothesize that gall-inducing species will show expansions in gene families related to nutrition and plant manipulation through specific developmental pathways, while inquiline and parasitoids lineages may display signs of selection relaxation and pseudogenization of some of these same orthogroups. Moreover, convergent genomic patterns across independently evolved gall-inducing lineages may reveal repeated evolutionary solutions to similar ecological challenges. By leveraging the multiple independent lifestyle transitions in Cynipoidea, this study provides a powerful framework to investigate genomic signatures underlying major ecological innovations. The results will offer key insights into the evolutionary mechanisms facilitating major lifestyle shifts and the extent to which convergent adaptations shape genomic evolution.



MBD2/3 lost its methyl-CpG binding ability in multiple families of Holometabola

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MEETING ROOM 113, August 19, 2025, 11:00 AM - 1:00 PM

DNA methylation is sparse in insects, compared to vertebrates. This reduction is even more pronounced in Holometabola, where the loss of DNA methyltransferases DNMT1 and DNMT3 has occurred several times. In some Holometabola, DNA methylation is lost entirely. Methyl-CpG-binding domain (MBD) proteins bind methylated CpGs and are therefore important readers of these epigenetic marks. We hypothesize that the evolutionary reduction of genome-wide methylation may be paralleled by changes to MBD proteins. Among insects, only a single MBD family member, MBD2/3, is known. Two isoforms of MBD2/3 have been identified in *Bombyx mori*, MBD2/3-L and MBD2/3-S. The long isoform MBD2/3-L contains a complete MBD domain spanning the first two exons, whereas the short isoform MBD2/3-S lacks the second exon and therefore half of its MBD domain. It has been reported that only

the MBD2/3-L isoform is able to bind methyl-CpGs.

In this study, we analyzed transcriptomic and genomic sequence data across holometabolous orders to identify MBD2/3 genes and their isoforms. Our findings reveal that MBD2/3 is highly conserved in sequence and gene structure. While both isoforms are present in most hemimetabolous orders, the long isoform MBD2/3-L, capable of binding methylated CpG, has been lost in multiple holometabolous orders. The results suggest that MBD2/3 has lost its ability to bind methyl-CpG in several insect orders, with several independent losses in Holometabola. This occurred through different changes to the MBD domain, from sequence divergence within the domain to the absence of half of the MBD domain. These losses may be linked to the reduced levels of CpG DNA methylation.



Olfaction evolving: a model of the evolutionary transition from deterministic to stochastic odorant receptor gene choice

Zug R¹, Martelli C¹, Kokko H¹

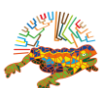
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MEETING ROOM 113, August 19, 2025, 2:00 PM - 5:00 PM

In both insects and vertebrates, odors are sensed by odorant receptors (ORs) in olfactory sensory neurons (OSNs). OR genes are one of the largest gene families in animals, but there is also high variability in OR gene number between species, with about 50 in *Drosophila* and about 1500 in the mouse. This diversity likely arose through independent gains and losses of OR genes along different lineages, in response to different selection pressures. According to the canonical “one neuron–one receptor” view, each OSN only expresses a single OR allele, which is remarkable given the high number of OR genes in animal genomes. Therefore, a crucial question is how a single OR gene is chosen to be expressed. In *Drosophila*, OR gene choice is thought to be deterministic, requiring a specific combination of transcription factors. In contrast, OR gene choice in vertebrates is stochastic: one OR allele that happens to lie in a supportive transcriptional environment is expressed, while all others are repressed. Within insects, ants show the largest number of OR genes, with around 500 in the clonal raider ant. Interestingly, recent evidence suggests that OR gene choice in ants is stochastic, unlike in *Drosophila* and as in vertebrates. It is likely that deterministic gene choice becomes unfeasible with a large number of OR genes, suggesting that, during

insect evolution, there was at least one transition from deterministic to stochastic gene choice, concomitant with OR gene expansion. Here we present a simple model of such a transition to better understand its regulatory underpinnings and evolutionary consequences.

In the model, we assume that, over evolutionary time, the number of relevant odors in the environment increases, and the olfactory system is under selection to keep pace with this increasing complexity by evolving appropriate ORs, through gene duplication and divergence. We also assume that OR gene choice is deterministic initially (with only a small number of odors present), but that the cost of deterministic gene choice becomes too high as the number of OR genes increases, so that stochastic gene choice gets favored. We are currently implementing and analyzing the model, and I will present modeling results and discuss them in light of recent empirical findings regarding OSN fate determination in insects and vertebrates. In particular, we expect the model to shed light on whether co-expression of several OR genes, as recently reported in insects, is a transient phenomenon during neuronal differentiation or whether it actually challenges a rigid “one neuron–one receptor” paradigm, endorsing the olfactory system with enhanced adaptability.



Origin of evolutionary innovations: from cell and developmental biology to trait utility and diversification.

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MEETING ROOM 113, August 19, 2025, 2:00 PM - 5:00 PM

Evolutionary innovations are lineage-specific traits that confer novel functions, often enabling access to previously unavailable ecological niches. Despite their prevalence, the developmental, cellular, and molecular mechanisms underpinning these traits—and the ecological forces that shape their evolution—are not well understood.

Here, we investigate the emergence of an evolutionary innovation using the propelling fans of *Rhagovelia* water striders as a model. Among the ~150 genera of Gerromorpha, *Rhagovelia* alone comprises over 20% of species and has radiated extensively into tropical fast-flowing streams—environments that dominate (>90%) aquatic habitats in these regions and present significant locomotory challenges.

We demonstrate that fan evolution in *Rhagovelia* is associated with the origin of a unique giant cell, absent in all other Gerromorpha. At the molecular level, the fan's development involves two distinct rounds of gene duplication: one through retrotransposition and the other via tandem duplication of fan-specific genes. Intriguingly, sister lineages that also possess fans—differing in number and morphology—lack both the giant cell and the associated genetic duplications.

Our findings reveal a clear example of how evolutionary innovations originate and diversify through novel cellular and genetic mechanisms, driving ecological expansion and lineage success.



Patterns of Gain and Loss of microRNA Families Explain Divergence in Mammal Pregnancy Phenotype

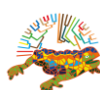
Fenn J¹, Edge J², Ovchinnikov V³, Amelkina O², Sinclair M¹, Mcinerney J⁴, Tinning H², Butt Z², Fromm B⁵, O'connell M¹, Forde N²

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MEETING ROOM 113, August 19, 2025, 2:00 PM - 5:00 PM

Diverse placental morphologies have evolved multiple times across mammalian lineages, resulting in huge variation of both taxon-specific and convergent traits concerning the structure and function of the placenta and other pregnancy morphologies. The evolution of regulatory systems associated with these traits is, however, largely unknown. MicroRNAs (miRNAs) are a group of regulatory molecules which show a high level of diversity in placental mammals, following a huge expansion of miRNA families at the advent of Eutheria. Their expression has been implicated in various developmental processes and pathologies associated with pregnancy. We identify presence/absence patterns of miRNA gene families across 300 high quality therian mammal genomes, highlighting distinct patterns of miRNA family gain and loss which associate with innovation of placental phenotype. By examining convergently-evolved phenotypes, we find evidence supporting some level of evolutionary determinism, whereby traits repeatedly

evolve as a result of convergent loss or preferential retention of specific miRNA families. Using multiple analytical approaches, we show that the placental phenotype is predictable from miRNA gene family repertoire. We find 30 miRNA gene families significantly associated with placentation phenotypes with targets enriched for development-associated biological processes. Furthermore, to test whether correlation is linked to causation, we demonstrate that MIR-11968 is specifically associated with the cotyledonary placental shape. We have confirmed expression of this miRNA in term placenta, with spatial differences in expression in another key tissue of mammalian pregnancy - the endometrium. Overall, this work demonstrates mammalian placental phenotypes are so strongly linked to genomic miRNA content, that miRNA repertoire is predictive of the phenotype, and further we demonstrate the causal effect of MIR-11968 on cotyledonary placental shape.



Predicting the functional impact of single nucleotide variants in *Drosophila melanogaster* with FlyCADD

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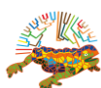
MEETING ROOM 113, August 19, 2025, 11:00 AM - 1:00 PM

Understanding how genetic variation shapes phenotypic diversity is a key challenge in evolutionary biology. Single nucleotide polymorphisms (SNPs) represent the most prevalent form of genetic variation and play fundamental roles in adaptation, fitness, and trait evolution. SNPs are often associated to traits in genome-wide association studies (GWAS). However, distinguishing causal from neutral variants remains a major obstacle. Genomic data on SNPs can be leveraged to understand the impact of mutations. With available genome editing techniques, and high-quality genomic data, *Drosophila melanogaster* is a good model organism to study the genotype-phenotype connections at single nucleotide resolution. We introduce FlyCADD, a computational tool predicting probability that SNPs will have functional impact in *Drosophila melanogaster*.

FlyCADD builds on the widely used Combined Annotation-Dependent Depletion (CADD) framework, originally developed on the human genome, and integrates over 650 genomic features—including conservation scores, GC content, and DNA secondary structure—into a single score reflecting a variant's predicted impact on evolutionary fitness. With FlyCADD, single nucleotide variants across the entirety of the *D. melanogaster* genome can be scored based on predicted functional impact. By leveraging high-quality *Drosophila* genomic annotations, FlyCADD provides a scalable

and systematic approach to evaluate genetic variants, whether naturally occurring or experimentally introduced. We demonstrate the utility of FlyCADD across several applications. First, we show that FlyCADD can prioritize phenotype-associated SNPs based on predicted functional impact, aiding in the identification of potentially causal variants from genome-wide scans. This has important implications for studies of microevolution, local adaptation, and complex trait variation in populations. Second, we apply FlyCADD to evaluate the functional significance of naturally segregating polymorphisms, refining our understanding of how standing genetic variation contributes to fitness differences. Finally, we illustrate how FlyCADD can be used to optimize CRISPR-Cas9 experimental designs by predicting the potential functional impact of engineered mutations, thereby improving experimental efficiency in functional validation studies.

By providing an evolutionarily informed framework for SNP impact prediction in fruit flies, FlyCADD enhances our ability to interpret (natural) genetic variation and link genotype to phenotype at nucleotide resolution, anywhere in the genome of *D. melanogaster*. This tool has broad applications for evolutionary genetics, functional genomics, and molecular evolution research, making it a valuable resource for both theoretical and experimental studies in *Drosophila*.



Template-switching and rapid emergence of novel gene regulators

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MEETING ROOM 113, August 19, 2025, 2:00 PM - 5:00 PM

Background: The function of several RNA-based gene regulators (such as microRNAs) relies on hairpin structures, which are formed after the transcription of inverted repeats in DNA sequences. We have recently shown that template-switching during DNA replication can generate not only inverted repeats but also functional miRNA loci. In this process, the replication machinery: 1) stalls and dissociates from the template strand, 2) associates with the nascent or complementary strand, 3) replicates a few bases on the incorrect strand, and 4) re-associates with the original strand. This occasionally results in the formation of a perfect inverted repeats that, if transcribed, can form RNA hairpin structures, which are fundamental structural elements in functional RNAs. In this study, we screened human RNA gene regions for historical template-switching events that created perfect inverted repeats. We show that several human microRNAs originated from a single template-switching mutation event in the primate lineage¹. In addition, our data suggests that template-switching has contributed to the formation of hairpins in other RNA genes, such as long non-coding RNAs.

Materials and Methods: We systematically screened all human RNA gene loci in multiple mammalian genome alignments containing predicted ancestral genome sequences. We systematically compared each child and parent sequence pair to identify insertions in the child sequence that could be explained by a template-switching event. Secondary structures were predicted for positive cases.

Results: Template-switching events were particularly common in microRNA genes (111 cases), where in 79 cases template-switching events fully explained a novel hairpin. Notably, the majority of template-switching origin microRNA hairpins were located within introns

of protein-coding genes, allowing the nascent microRNAs to acquire transcriptional capacity immediately. Most of the novel template-switch origin microRNAs (57) arose in the root of primates, 11 in the root of Old world monkeys and 5 in the root of apes. The rest only affected a subset of apes. In addition, we screened all ancient template-switching events within the human introns and identified over 50,000 such cases, of which 6,000 had created microRNA-like inverted repeats, suggesting that only a small proportion of hairpins evolve into functional entities. We also identified novel hairpin structures from other RNA genes, such as long non-coding RNAs, although these were less frequent suggesting that the hairpin structures in lncRNAs are not as essential as miRNAs.

Conclusions: Template-switching has the potential to rapidly generate inverted repeats in DNA, which can form novel RNA hairpin structures in transcribed regions. Under favourable conditions, template-switching can lead to the rapid emergence of new gene regulators. Although template-switching is relatively frequent, only a small number of these events ultimately lead to functional entities. Nevertheless, as several gene regulators (e.g. microRNAs) are based on inverted repeats in the genome, this mechanism may provide material for the rewiring of gene regulation and the rapid adaptation of organisms to changing environments.

1. H.A.M. Mönttinen, M.J. Frilander & A. Löytynoja. Generation of de novo miRNAs from template switching during DNA replication, *Proc. Natl. Acad. Sci. U.S.A.* 120 (49) e2310752120, <https://doi.org/10.1073/pnas.2310752120> (2023).



The epigenetic landscape across Lepidoptera and its impact on chromosomal rearrangements

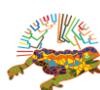
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Holocentric chromosomes are defined by lacking a single centromere region, instead, they present multiple centromere-like structures, and have independently evolved in multiple lineages of plants and invertebrates. Due to this structure, chromosomal rearrangements like fusions or fissions are common in holocentric species, which can act as reproductive barriers and promote speciation. Groups presenting holocentrism as Lepidoptera, the order comprising moths and butterflies, displays and a high diversity in karyotypes, which is not equally distributed among and even within genera. This diversity may be due to the impact of large-scale rearrangements as a barrier to gene flow at different evolutionary scales. Former studies suggest that fusion

and fission sites are enriched for repetitive elements, which often have unique methylation patterns. However, the methylation landscape in Lepidoptera has remained largely unknown. Here, we characterise the methylation landscape in Lepidoptera and their association with genomic breakpoints underlying chromosomal fusions or fissions. For that, we used Bisulfite sequencing across 60 species of butterflies and moths to analyze genome-wide methylation patterns across the genome and in rearrangements breakpoints. Our results will uncover the relationship between the methylome and chromosomal rearrangements, shedding light on the epigenetic mechanisms associated with holocentric speciation.



The Escherichia coli genome is relatively devoid of cryptic promoters and repressor binding sites

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Prokaryotic promoters are DNA sequences which demarcate transcription start sites. Previous studies in *E. coli* demonstrate that promoters are relatively “simple”, occurring in ~10% of randomly synthesized DNA and emerging from single point mutations in non-promoter DNA. These findings raise the question: does selection account for spontaneous promoter emergence within prokaryotic genomes? Here, we first quantify the promoter activities of 17,129 random and 92,869 genomic DNA sequences using a massively parallel reporter assay (Sort-seq). We find that genomic sequences are about twice as likely to have promoter activity relative to random DNA, with many intragenic regions exhibiting stronger activity than random promoters. Second, we assay how new promoters emerge de-novo from 60

random and 225 genomic DNA sequences in ~700,000 individual mutants. We find that de-novo promoters are about three times more likely to emerge from point mutations in random DNA compared to genomic DNA. This emergence bias is due to the *E. coli* genome’s under-enrichment of both cryptic promoter sequences and transcription factor binding sites. While this evolutionary bias hinders spontaneous promoter emergence in the genome, it simultaneously makes de-novo genomic promoters stronger than random promoters, as they are less repressed. Such neofunctional biases may be a product of selection and have a profound impact on the evolution of gene expression and the emergence of de-novo genes.



The Role of Gene Copy Numbers, Gene- and Isoform-Expression on Cuticular Hydrocarbon Diversity across Hymenoptera species

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Insects possess a cuticular hydrocarbon (CHC) layer on their cuticle that serves both as barrier against desiccation and as medium for chemical communication. CHC profiles are highly variable, often differing markedly even between closely related species. In Hymenoptera, these profiles vary not only among species but also within species, across sexes, and in social species also between castes. Between-species differences typically involve distinct compounds or compound classes, while within-species variation mainly concerns differences in the relative abundance of shared compounds.

Previous genomic studies suggest that CHC-synthesis gene families vary in copy number among species, with social species exhibiting expanded gene families relative to solitary ones. Based on this information, we hypothesized that species-specific CHC profiles are shaped by lineage-specific gene copies, while within-species variation (between sexes and castes) is primarily driven by the level of gene expression and alternative splicing.

To investigate the importance of gene expression as well as gene and isoform usage between species, sexes and castes, we sequenced the transcriptomes and isoforms from males and females--or queens and workers--of five wasp, bee and ant species. We compare gene family copy numbers and examine whether orthologous genes are expressed across species with convergent CHC profiles. Within species, we report on differences in gene usage, expression levels and isoform diversity across sexes and castes.

Our results shed light on the role of gene family evolution in mediating between species-level CHC diversity, and the importance of transcriptional and post-transcriptional regulation for within species CHC diversity.

This integrative approach ultimately allows elucidating the contribution of gene family evolution and gene regulation to the diversification of a highly plastic key ecological and social trait in insects.



The role of transposable elements in the adaptation of amphipod crustaceans to the deep sea.

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MEETING ROOM 113, August 19, 2025, 2:00 PM - 5:00 PM

Background: Theory predicts that the few founding individuals of a colonising population will experience a bottleneck and have reduced genetic diversity. So, how is it that populations can rapidly adapt and diversify when they colonise a novel or challenging environment? Transposable elements (TE) may help reconcile this paradox. TE are mobile sections of DNA that are highly mutagenic. Typically, TE mobilisation is detrimental to the host, and TE are therefore suppressed with epigenetic mechanisms such as CpG methylation. However, in rare cases, TE mobilisation can be beneficial for the host organism by generating mutations and increasing genetic diversity.

The extreme deep sea is an exciting opportunity to explore the beneficial role of TE in the colonisation of a new, extreme environment. There is an environmental and physiological bottleneck at the bottom of the Bathyal zone (ca. 4000m below sea level). Despite the extreme environmental conditions, amphipod crustaceans colonised beyond this after the last deep-sea mass extinction and became the dominant macrofauna at abyssal and hadal depths. Deep-sea amphipods show genomic obesity (5-35Gb) and expansion of key gene families such as heat shock proteins (HSP), which play a crucial role in protein folding under extreme hydrostatic pressure. We hypothesise that when transitioning to the abyssal, environmental stressors caused a breakdown of CpG methylation policing of TE, resulting in an explosion of TE activity, which generated the evolutionary novelty required for amphipods to colonise the deep sea, for example, by increasing the copy number of HSPs.

Materials and methods: A new draft genome assembly of *Hirondellea gigas*, from 8847m below sea level in the Mariana Trench, was

generated and compared to the genome of the model shallow-marine amphipod *Hyaella azteca*. Here, we use comparative genomics, epigenomics and transcriptomics to assess the role of TE in genome evolution, HSP evolution and HSP70 copy number increase, and TE control by CpG methylation.

Results: TE have increased the genome size of *H. gigas*, with 45% of the 12.86Gb *H. gigas* draft genome assembly being TE. Only 25% of the 0.55Gb genome of *H. azteca* is from TE. TE in *H. gigas* are likely not controlled by CpG methylation, with only 0.472% of CpGs being methylated and an abundance of young and active TE.

H. gigas has unique isoforms of HSP70 and 90 that are under purifying selection and are not found in shallow-water species. All HSPs in the *H. gigas* genome are in TE-dense regions of the genome; this is not the case in *H. azteca*. TE likely duplicated HSP70s in *H. gigas*; there are 11 functional copies of HSP70 in *H. gigas*, versus 8 in *H. azteca*. Three HSP70 paralogs are detected in TE-HSP repeat units, suggesting this unit mobilised as one, generating segmental duplications. Five other HSP70 paralogs were likely duplicated in tandem as a result of TE-mediated structural variation.

Conclusions: The amount, diversity and dynamics of TE in *H. gigas* are consistent with TE activity facilitating adaptive evolution to the deep sea. The data are consistent with the proliferation of TE following the breakdown of CpG methylation policing. The proximity and association of heat shock proteins with TE implies a route for adaptive evolution to high hydrostatic pressure through gene family expansion. This work adds to a growing body of evidence for the role of transposable elements in adaptive evolution.





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S30 - Genomic insights into evolutionary adaptation and species movements in a changing climate



Analysing the Evidence for a Seasonal Migration in *Panacea prola* Using Population Genomics

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¹Queen Mary University Of London, ²Natural History Museum, ³Pontifical Catholic University of Peru

POSTER SESSION 3 - **P03.093**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Mass-movements of insects constitute large movements of biomass, which can have important economic and ecological implications. The tropics harbour the majority of the Earth's insect biodiversity, yet our knowledge of species movements in the tropics is lacking in comparison to our knowledge of insect migrations in temperate areas. This is in spite of the fact that many habitats within the tropics show marked seasonality in climatic conditions. As climate change reduces the predictability of environmental cues that may trigger adaptive behaviours such as migration, and land-use change leads to unprecedented biodiversity losses, it is important to document and understand tropical insect migrations. Understanding how Lepidoptera may show seasonal plasticity through migration behaviour and shifting the timing of their development and life history events, will provide insight into how rapidly butterflies may be able to adapt to future climatic conditions.

Here, we study *Panacea prola* (Doubleday 1848), an Amazonian butterfly in the family Nymphalidae, which has been observed making directional mass-movements during the dry season each year between 2019 and 2024, at the research station Finca Las Piedras in Southeast Peru (Gallice et al., 2020 [preprint]). This has led to the hypothesis that *Panacea prola* may be migrating to avoid unfavourable climatic and environmental conditions during the dry

season. This study aims to examine the evidence for a seasonal migration in *P. prola*. Specifically, we:

1) test how wing morphology and reproductive output differ between individuals presumed to be migratory and those collected outside the migration window.

2) Compare isotopic signatures of butterflies between different study sites, and analyse how this reflects local vs non-local origin.

3) Quantify demographic parameters using whole-genome sequencing. Whole genome sequencing of individuals from populations in Southeast Peru will be used to model patterns of gene flow, using site frequency spectrum approaches. Understanding the predominant directions of gene flow may shed light on the direction of individual movements and whether there is a return movement in *P. prola*'s seasonal flight.

The extent of population structuring will also be assessed, with the expectation that little population structuring will occur if the butterflies are readily dispersing or migrating between sites. These population genomic approaches will inform us of whether, and to what extent, butterflies may be migrating or dispersing between sites, with implications for conservation measures.

4) We will additionally investigate whether adaptive genetic variation may contribute to differences in phenotype between migratory and non-migratory individuals.



Evolutionary history of the rapid post glacial diversification in the songbird genus Junco

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POSTER SESSION 3 - **P03.097**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Post glacial radiations offer a near real time window into speciation dynamics following abrupt climate shifts. The radiation of Dark-eyed Junco (*Junco hyemalis*) forms following the Last Glacial Maximum (LGM) provide a unique system to understand the relative roles of selective and neutral factors involved in rapid speciation, yet the demographic bridge between ancestral refugia and modern diversity remains unresolved. Despite its spectacular color and behavioral variation, the Junco complex lacks a genome scale synthesis that connects the yellow eyed junco expansion with the subsequent burst of divergence inside the dark eyed junco lineage. Here we ask: How did post LGM range shifts, gene flow and changes in population size interact to generate today's latitude graded diversity? We performed genome resequencing of 225 birds covering 19 junco taxa and paired these data with CHELSA TraCE21k paleoclimatic niche models. Coalescent species tree analyses recover a ladder like topology that tracks latitude and points to a single north bound colonization ~18 kya from a Mesoamerican refugium. Principal

components and ADMIXTURE analyses delineate a shallow genomic cline punctuated by sky island breaks and the low elevation desert basins separating them, while D statistics reveal distance decaying bidirectional gene flow culminating in a recent admixture hotspot in the northern Rockies. Fastsimcoal2 analyses favor divergence with gene flow over strict isolation and, together with SMC++, identify serial founder bottlenecks followed by a five-fold increase in effective population size between 17 and 9 kya. Ecological niche models reconstruct a continuous montane corridor from Mexico's Sierra Madre to the Madrean Sky Islands of the SW USA throughout the late glacial, neatly predicting the genomic contact zones uncovered here. Our results portray the Junco radiation as a climate-driven serial founder cascade: limited but persistent introgression, rapid demographic rebound and moving habitat corridors forged marked phylogeographic structure within ten millennia, directly linking the Yellow-eyed Junco expansion to the explosive, ongoing diversification of Dark-eyed Juncos across North America.



Genome assembly of pulmonate snail *Ellobium chinense* illuminates genomic adaptation and contrasting historical demography

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POSTER SESSION 3 - **P03.091**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Ellobium chinense, an amphibious pulmonate snail inhabiting brackish estuarine salt marshes in the northwestern Pacific, is listed as Vulnerable in Korea and Japan due to severe habitat loss. As a transitional species bridging marine and terrestrial environments, *E. chinense* offers a valuable model for understanding genomic adaptations to fluctuating intertidal ecosystems. However, genomic resources for this species have remained unexplored. To investigate genetic adaptations associated with habitat transitions, we generated a draft genome of *E. chinense* and conducted comparative genomic analyses with three other gastropod species representing marine (*Aplysia californica*), freshwater (*Biomphalaria glabrata*), and terrestrial (*Achatina fulica*) habitats. We further re-sequenced whole genomes of 14 individuals from China, Korea, and Japan, followed by demographic inference using

MSMC2. Comparative analyses identified 1,019 *E. chinense*-specific orthogroups enriched in GO terms related to cell adhesion, ion transport, DNA binding, and synaptic transmission—functions potentially linked to adaptation to osmotically dynamic environments. Population genomic analyses revealed contrasting demographic trajectories across the three northwestern regions during the last glacial period, likely shaped by region-specific responses to glacial–interglacial climatic fluctuations. This study provides the first genomic insight into *E. chinense*, highlighting its molecular adaptations to intertidal, terrestrial habitats and uncovering contrasting population histories influenced by Quaternary environmental change. This work was supported by the management of Marine Fishery Bio-resources Center (2025) funded by the National Marine Biodiversity Institute of Korea (MABIK)



Genomic Legacy of the Ice Ages

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POSTER SESSION 3 - P03.096, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Climate change is one of the greatest threats to biodiversity today, pushing species to adapt, shift their ranges, or face extinction. However, this is not the first time life on Earth has faced dramatic climatic fluctuations. During the Ice Ages, repeated cycles of glaciation and warming reshaped landscapes, species distributions, and evolutionary trajectories. Studying how species responded in the past can provide critical insights into their future responses.

European hares are an excellent case study for understanding climate-driven range shifts and adaptations. Their historical distributions were strongly shaped by glacial cycles, which promoted multiple interspecific hybridization events. Among them, two species stand out: the broom hare (*Lepus castroviejoi*) and the Italian hare (*Lepus corsicanus*). Despite being geographically isolated in distinct southern European mountain ranges, both species carry mitochondrial DNA from the cold-adapted mountain hare (*Lepus timidus*), rather than from their own nuclear backgrounds.

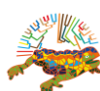
This unexpected mitochondrial pattern suggests ancient hybridization with the mountain hare during glacial periods when shifting ranges allowed contact. The subsequent fixation of these mitochondrial

lineages indicates that these interactions were not merely transient.

Materials and Methods: To investigate this further, we analyzed whole-genome data from all European hare species, using a combination of Hidden Markov Models and Neural Networks to detect genomic regions of mountain hare origin introgressed in both the broom and Italian hares. To infer the timing and extent of introgression events, we also constructed dated phylogenies including data from all European hare species.

Results: We identified genomic regions of likely mountain hare origin in both species. Some of these regions may be under positive selection, hinting that they helped these hares persist through the dramatic range and temperature shifts that marked the end of the last Ice Age.

Conclusion: These two species are more than evolutionary outliers – they offer unique insights into the long-term consequences of hybridization, climate shifts, and isolation. As climate change accelerates, understanding these historical legacies may help inform conservation strategies for the future.



Genomics of northern adaptation: Prevalence and mode of selection in northern *Arabidopsis lyrata* populations in Eurasia

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POSTER SESSION 3 - **P03.090**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The ability of species to adapt to harsh environments has enabled them to expand into newly available habitats, such as those that emerged after the last glacial maximum. Northern Europe, for example, was colonized by numerous species less than 20,000 years ago following the retreat of the ice sheets. This makes it an ideal natural laboratory for studying adaptation to extreme conditions at high latitudes. The plant species *Arabidopsis lyrata* successfully spread across Eurasia to high latitudes, likely through parallel processes. Understanding the molecular basis of such parallel adaptations is of great interest to evolutionary biologists and has important implications for conservation and food security. In the context of climate change, uncovering the genetic mechanisms behind adaptation to high latitudes is especially relevant, as many species are expected to shift northward in response to rising temperatures.

Adaptation to high-latitude environments is primarily characterized by novel phenotypic response, for example to a new combination of day length and temperature. Plants need to cope with long cold winters, short growing seasons, and strong annual fluctuations in day length. Selection acting on the genetic variants responsible for such traits will – due

to linkage disequilibrium – leave a traceable footprint around the selected variant, allowing to detect larger areas around the variants, a pattern known as a selective sweep. Thanks to the fast-developing computational and methodological approaches to identify footprints of selection even in a complex genetic background but this requires also carefully designed sampling setup. In this study, we aim to build a population genetics model to advance understanding on high latitude adaptation by compiling a large population re-sequencing collection of the model species *A. lyrata* populations from central Europe, Great Britain, Iceland, Scandinavia and Siberia. By utilizing this dataset, we will investigate the intertwined demographic and adaptive processes using population genetic approach. We will specifically utilize the parallel northward spread setup of this species and compare the different branches of high latitude colonization. The results of this project will help to understand how species adapt to extreme environments, how parallel these adaptations might be and due to the retraction of the ice sheets, we can put these evolutionary processes into a timeframe of ~20,000 years.



Polar bear evolutionary adaptation: insights from non-coding accelerated regions

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POSTER SESSION 3 - **P03.204**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Polar bears are an ideal system to understand selective pressures as populations adapt to new environments. Their ecological niche shifted radically from a generalist temperate omnivore to a specialist arctic carnivore, subsisting primarily on seal fat, making polar bears a unique evolutionary model for arctic adaptation. Prior studies of positive selection on protein coding sequences have identified selection on genes involved in cholesterol metabolism. However, selection on non-coding gene regulatory elements, the most common target of positive selection, has not been explored. In this study we analyzed whole genome sequence data from 85 bears to identify Accelerated CREs in polar bears, across 847830 regions orthologous to human CREs documented by ENCODE and annotated using the Zoonomia 241 species alignment. We included two ancient polar bears to distinguish between selection before and after the time of the ancient samples (70-130 kya). Because of the challenges posed by incomplete lineage sorting (ILS) and admixture among polar bears and brown bears, we expand upon the traditional AR detection framework to

include population level comparisons. This allows for better detection of positive selection during what we might term intermediate evolutionary time spans, too long for selective sweep-based assays but too short to be unaffected by ILS and admixture. Here we identified 790 accelerated regions as non-coding targets of positive selection in polar bears. Using two ancient polar bear samples, we were also able to distinguish between recent adaptations and adaptations that were already present in ancient samples. We found significant enrichment for selection on critical gene regulatory systems in polar bears including insulin resistance, inflammation and lipid metabolism, fatty acid degradation, adipogenesis and pigmentation. Globally our findings show that selection is acting on pathways that represent important adaptations to the arctic environment such as growth, development, and immunity. This data presents an exciting and novel opportunity to identify the metabolic innovations that allow polar bears to tolerate an extremely high-fat diet, substantially improving knowledge of how species adapt to new climate niches.



Twenty years of monitoring and common garden experiment revealed a possible difference in heat tolerance under climate changes and robust species barriers between sympatric sticklebacks

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POSTER SESSION 3 - **P03.092**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Climate change can change species composition and possibly induce hybridization. Here, we tested this hypothesis using a sympatric species pair of *Gasterosteus aculeatus* and *G. nipponicus* in eastern Hokkaido, Japan. Water temperatures in this region have recently increased due to global warming. Sticklebacks are coldwater fish distributed in the northern part of the Northern hemisphere, so they will be influenced by global warming. However, it remains elusive how the species composition, their life histories, and hybridization rates have changed under climate changes.

We took advantage of the historical samples collected in the last 20 years in the sympatric habitat of these two species, Shiomi River in eastern Hokkaido. We applied stable isotope analysis and genomic analysis to reveal the changes in life histories, species composition, and hybridization rates. We also conducted laboratory experiments to reveal the difference in thermal tolerance between the two stickleback species.

Firstly, we conducted laboratory rearing experiments to show that *G. aculeatus* showed lower tolerance to high salinity compared to *G. nipponicus*. Consistent with this result, stable isotope analysis showed a trend that the number of anadromous *G.*

aculeatus has declined in recent years, while there are resident *G. aculeatus* until now. Climate change may influence the life-history strategy of *G. aculeatus*.

ddRAD-seq analysis of the 20 year samples showed that *G. nipponicus* migrated into the river only in limited years and hybrids appear in a few years after that event, but the introgressed *G. nipponicus* alleles were rapidly purged within a few years. Sex identification showed that most F1 hybrids resulted from matings between female *G. nipponicus* and male *G. aculeatus*. Previous research has shown that male hybrids from this cross are sterile and the reciprocal hybridization is rare due to sexual isolation. Thus, our results are consistent with these previous findings, which suggests that both pre-zygotic and post-zygotic reproductive isolation mechanisms seem to persist.

In conclusion, climate change might possibly drive the reduction in anadromy in *G. aculeatus*. Future increase in water temperatures would exceed physiological thresholds of *G. aculeatus*. Nevertheless, the species barriers seem to be still present between the species. This study highlights both the impact of climate changes and robustness of reproductive isolation.



When Isolation echoes: Tracing Temporal Genetic Drift and the Loss of Genetic Diversity in Baetican Toothcarps (*Aphanius baeticus* Doadrio, Carmona & Fernández-Delgado, 2002)

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POSTER SESSION 3 - **P03.094**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Freshwater fish species are facing an alarming global decline within the ongoing changing climate context, marked by reductions in both population size and genetic diversity. This crisis is especially severe in human-altered landscapes such as the Iberian Peninsula, where artificial barriers fragment habitats and restrict gene flow. Endemic species from this region, like the endangered Baetican Toothcarp (*Aphanius baeticus* Doadrio, Carmona & Fernández-Delgado, 2002), are particularly vulnerable. Restricted to a narrow range along Spain's South Atlantic coast, this species offers a valuable model to assess how isolation and environmental change shape genetic structure and local adaptation over time.

We conducted a temporal genomic study based on two comprehensive sampling campaigns covering the species' full distribution: one in 2008 and another in 2022, roughly seven generations apart. Using genome-wide SNP data, we analyzed changes in population structure, connectivity, and genetic diversity. Analyses including phylogenetic trees, PCA, Structure, Admixture, and gene flow modeling revealed that most major genetic clusters remained stable across time. However, subtle genetic differences were detected in all previously defined genetic units, pointing to a progressive loss of genetic variation.

Beyond genetic structure stability, suggesting well-adapted genotypes, quantitative metrics offered a clearer view of ongoing genetic erosion. Nucleotide diversity (π), effective population size (N_e), and

absolute divergence (d_{xy}) declined consistently between 2008 and 2022. Meanwhile, pairwise F_{st} and clustering patterns showed more complex changes, suggesting increased differentiation rather than recovery. Analyses of heterozygosity and Runs of Homozygosity (ROH) further supported the presence of incipient bottlenecks evidenced by reduced rare alleles and increased homozygosity in some populations.

We also recorded a marked reduction in the number of individuals sampled across most locations and the likely disappearance of at least one population. These patterns suggest that the loss of genetic diversity is not uniform across the species' range, but more pronounced in smaller or more isolated units, most vulnerable to the global warming impact. Importantly, the temporal dimension of our approach allowed us to identify emerging patterns that might otherwise go unnoticed in single-timepoint studies.

Altogether, our results provide a temporal perspective on the genomic consequences of habitat fragmentation, drought intensification, and demographic contraction in an endangered freshwater fish. The findings highlight the urgency of conservation efforts aimed at preserving connectivity, protecting genetic diversity, and securing the long-term persistence of distinct population units. Temporal genomic data, such as those presented here, offer a powerful tool for early detection of critical genetic shifts and timely conservation intervention.



A genomic perspective on population structure and adaptation to aridity in the four-striped mouse *Rhabdomys bechuanae* in Southern Africa

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MEETING ROOM 120+121, August 21, 2025, 2:00 PM - 5:00 PM

Elucidating the evolutionary processes driving evolution of living organisms in dry environments is central to understanding adaptive responses to climate change. The genomics of adaptation to dry environments is a rapidly growing research field, but its application to non-model organisms that face increasing aridity remains limited. We addressed this gap by applying RAD sequencing and population genomics analyses to 230 individual samples of the four-striped mouse species *Rhabdomys bechuanae*, a species distributed across semi-arid to hyper-arid habitats in southern Africa – an area projected to experience intensified aridity in the next century. Combining demographic inference, population structure analysis, and genotype-environment association approaches, this study addresses past and current genetic variation within this species in relation to past and present-day variation in aridity, as well as the genetic basis of adaptation to aridity. Our demographic inference analysis suggests a sharp decline in effective population size since 15 kya, coinciding with a long-term increase in regional aridity; this decline may reflect reduced population connectivity in periods of increased aridity. We also show that the current population genetic structure of the species follows a pattern of isolation by distance that is correlated with genetic variation along an

aridity gradient. Genotype-Environment Association analysis, accounting for this population structure, revealed SNPs that were significantly associated with the aridity-related environmental variable (Aridity Index), providing evidence for the genetic adaptation of *R. bechuanae* to variable degrees of aridity across its area of distribution. These SNPs map to genes involved in nucleic acid modification, osmoregulation and neurotransmitter transport. Functional enrichment analysis further highlights functions affecting cell machinery and cytoskeleton regulation. These mechanisms could be targets of selection in arid environments due to their physiological importance in the face of osmotic stress and resource (water/nutrient) scarcity. This polygenic basis of adaptation to aridity in the four-striped mouse is largely consistent with patterns observed in other arid-occurring rodents (e.g. *Dipus sagitta*, *Dipus sowerbyi*, *Peromyscus eremicus*, *Meriones meridianus*, *Phodopus roborovskii*), which highlights potential shared mechanisms of adaptation to dry environments. Our study demonstrates the power of integrative population genomics to uncover adaptation in non-model species, offering key insights into the genomic underpinnings of resilience to aridity under global climate change.



Adaptation, and genetic substructure of Atlantic Cod (*Gadus morhua*) in a changing climate

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MEETING ROOM 120+121, August 21, 2025, 2:00 PM - 5:00 PM

Identifying and understanding intraspecific diversity is fundamental to managing living systems under climate change. Among marine taxa, this diversity underlies climatic and oceanographic adaptations, life history, and migratory variation. Genomic sequencing has enabled the matching of individual behaviors such as migration, and adaptations such as thermal tolerance, and genomic variation across large spatial scales. Using this technology, it is now possible to identify the genomic architecture of climate-resilient traits and behaviour within marine species, and predict how this variation may be impacted under anthropogenic climate change.

Atlantic Cod (*Gadus morhua*) represents one of the most historically important marine species across the Atlantic, and has undergone dramatic declines resulting in fishery closures and ecological change. Significant diversity has also been identified within Atlantic Cod at both genomic and phenotypic levels. Abiotic variation in temperature and salinity, as well as behavioral variation between migratory and inshore groups with varying spawning times indicate substantial diversity with potential

for differing responses to disturbance. At the genomic level, large chromosomal inversions have been identified exhibiting varying levels of local and trans-Atlantic divergence and genomic parallelism, and corresponding to variation in ecology and behavior. Similarly, recent studies have also begun to identify polygenic patterns of adaptation and response to overharvest, together providing evidence of multiple levels of genetic diversity and resilience within this species.

Here, we conduct large scale whole genome resequencing in Atlantic Cod (*Gadus morhua*) in the Northwest Atlantic to understand how population structure driven by behavioural and climatic differences are arrayed spatially and temporally. We describe a new 1000 genomes dataset spanning sequences 1993 to present, allowing comparisons of pre and post fisheries collapse genetic structure. We explore genetic groups driven by spawning-associated genes as well as chromosomal inversions, identify environmental associations with this structure, and assess the implications of past and future climate change on the stability of Atlantic Cod.



Cis-regulatory divergence and heritable plasticity underlie seasonal adaptation in a tropical butterfly

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MEETING ROOM 120+121, August 21, 2025, 2:00 PM - 5:00 PM

Rapid climate change is reshaping seasonal environments and threatening the synchrony of ecological interactions. Understanding the genomic basis of phenological plasticity is critical for predicting species' responses and resilience to shifting climates. A key question is whether such plasticity has a heritable basis upon which selection can act. In the Afrotropical butterfly *Bicyclus anynana*, individuals develop into season-specific morphs with striking differences in wing pattern, life history, and behaviour. These seasonal forms are triggered by temperature cues that predict the onset of dry or wet seasons. Using whole-transcriptome data from abdomen tissue of individuals reared under controlled seasonal conditions, we investigate the regulatory mechanisms underlying this phenotypic plasticity. We show that co-expression network architecture is largely conserved across environments, suggesting a robust, canalised wiring of regulatory modules. However, differential expression between seasons is widespread, and two modules emerged as uniquely expressed in wet

season forms - enriched for thermosensory behaviour, mating, and learning functions, all key to seasonal fitness strategies. Notably, 24% of environmentally responsive genes and 47% of genotype-by-environment (G×E) genes are under cis-regulatory control - significantly more than expected by chance. These genes are enriched for network hub genes and located in regions of elevated genomic differentiation, indicating that cis-regulatory variants mediate heritable, fine-tuned responses to seasonal conditions. Our findings demonstrate that *B. anynana* harbours a complex, partially heritable regulatory basis for phenological plasticity. This provides a mechanistic framework for how seasonal organisms can respond to climate-driven shifts in environmental timing. By linking cis-regulatory architecture to adaptive phenotypic variation, our study highlights the potential for evolutionary resilience in the face of phenological mismatches and informs forecasts of species' vulnerability under future climate scenarios.



Climate adaptation and genomic offset in north Atlantic ptarmigan

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MEETING ROOM 120+121, August 21, 2025, 2:00 PM - 5:00 PM

Global environmental changes are occurring at an unprecedented pace, necessitating a deeper understanding of how species are differentially adapted to their ecosystems and how they may respond to climate shifts. In this study, we investigated the genomics of two closely related non-migratory birds, the Rock Ptarmigan (*Lagopus muta*) and the Willow Ptarmigan (*Lagopus lagopus*), across populations in Europe and North America. These species are significant as game birds in some regions and are considered severely threatened in others.

By analyzing whole-genome resequencing data from 99 individuals across their ranges and integrating local environmental variables from CHELSA and WorldClim, we explored patterns of genetic diversity, local adaptation, and predicted genomic offset under future climate scenarios.

Our results reveal that subpopulations of these species exhibit varying levels of vulnerability to predicted environmental changes. Specifically, lower genetic diversity was generally associated with a reduced ability to adapt to significant environmental shifts. We identified climate-associated single nucleotide polymorphisms (SNPs),

indicative of polygenic adaptation to climate; however, the specific SNPs varied between populations. Gene ontology enrichment analysis highlighted processes related to neurogenesis and general development, suggesting that both behavioral and physiological responses play roles in adaptation to environmental change.

Model projections identified Rock Ptarmigan populations from Greenland, Svalbard, and the Alps as being at the greatest risk from genomic offset, while Willow Ptarmigan populations in Norway and Newfoundland also exhibited elevated offset. Changes in temperature and precipitation emerged as the strongest predictors of population risk, though predictions of offset varied significantly between environmental models. These findings underscore the importance of testing across multiple models to establish a risk consensus.

In summary, we present an integrated approach for predicting population-level vulnerabilities across time and space by combining genomics and environmental data, offering a valuable framework for conservation efforts.



Convergent adaptation to latitude in fish

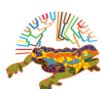
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MEETING ROOM 120+121, August 21, 2025, 2:00 PM - 5:00 PM

Understanding the repeatability of adaptation to climate is a central question in evolutionary biology, especially as global warming imposes similar selective pressures on numerous species worldwide. Previous studies have found evidence of convergent local adaptation to climate and global adaptation in distantly related conifers and plants (Yeaman et al., 2016; Whiting et al., 2024; Nocchi et al., 2024). Here, we investigated the genomic basis of adaptation to latitude, a proxy for climate, in two distantly related fish: Chinook salmon (*Oncorhynchus tshawytscha*) and threespine stickleback (*Gasterosteus aculeatus*). These species differ markedly in ecology, population structure, and life-history strategies, and have been separated by about 219 million years of evolution. We generated high-coverage whole-genome sequences for 137 Chinook salmon and 514 threespine sticklebacks sampled across broad latitudinal gradients, spanning 45 degrees across their full geographic range. Using Gene-Environment Association (GEA) analyses, we identified candidate genes strongly associated with latitude in each species, highlighting genomic regions likely involved in climate adaptation. We then compared these

candidate genes across species, focusing on shared orthologous groups of genes. Strikingly, we found that approximately 10% of those candidate genes are shared between the two species at the orthogroup level. This significant overlap indicates a high degree of repeatability in the genomic targets of climate adaptation, despite the deep evolutionary divergence and ecological differences between Chinook salmon and threespine sticklebacks. Our findings suggest that particular genes and pathways may be consistently recruited during adaptation to similar climatic gradients in fish. Besides, our sampling design for sticklebacks, spanning three independent latitudinal gradients, allows comparisons of parallel adaptation at both intra- and inter-specific levels. Overall, our study shows that certain genomic regions are repeatedly involved in climate adaptation, suggesting that evolution can be both predictable and constrained. This repeatability enhances our ability to forecast species' responses to climate change and highlights the value of comparative genomics for identifying adaptive genes as conservation-relevant targets in the face of climate-driven range shifts.



Evolutionarily distinct lineages of a migratory bird of prey show divergent responses to climate change

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MEETING ROOM 120+121, August 21, 2025, 2:00 PM - 5:00 PM

Accurately predicting species’ responses to anthropogenic climate change is hampered by limited knowledge of their spatiotemporal ecological and evolutionary dynamics. We combine landscape genomics, demographic reconstructions, and species distribution models to assess the eco-evolutionary responses to past climate fluctuations and to future climate of an Afro-Palaeartic migratory raptor, the lesser kestrel (*Falco naumanni*). We uncover two evolutionarily and ecologically distinct lineages (European and Asian), whose demographic history, evolutionary divergence, and historical

distribution range were profoundly shaped by past climatic fluctuations. Using future climate projections, we find that the Asian lineage is at higher risk of range contraction, increased migration distance, climate maladaptation, and consequently greater extinction risk than the European lineage. Our results emphasise the importance of providing historical context as a baseline for understanding species’ responses to contemporary climate change, and illustrate how incorporating intraspecific genetic variation improves the ecological realism of climate change vulnerability assessments.



Implications of expanding hybrid wood ant populations on heat tolerance and phenology under climate change

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MEETING ROOM 120+121, August 21, 2025, 2:00 PM - 5:00 PM

Climate change challenges all species. With rising temperatures and an increased frequency of extreme events such as heat waves and droughts, organisms are shifting their distribution to more suitable habitats. In turn, this will lead to new contact zones between previously isolated populations and thus increase hybridisation rates. However, our understanding of the extent and persistence of hybridisation and its impact on heat tolerance and phenology is limited. Here, we use mound-building wood ants, keystone forest species, and their naturally occurring hybrids to assess whether the extent of hybridisation has increased over the past 20 years and to study the implications of hybridisation for heat tolerance and phenology. We use whole-genome data from 170 populations collected in 2005 (N=78) and in 2023 (N=92) as well as life-history and long-term ecological data on wood ants and focusing on hybrids and their

cold-adapted parent *Formica aquilonia*. We show that (i) hybrids have persisted, slightly increased in number, and moved northward in Finland, and that (ii) hybrids perform better under heat stress and become active earlier in the season compared to the cold-adapted parent *F. aquilonia*. These results reveal that (i) the extent of hybridisation in wood ants is higher than previously estimated, with hybrids persisting over 20 years and even expanding their distribution area. Furthermore, (ii) hybrids likely have an adaptive advantage in terms of heat tolerance and phenology compared to their cold-adapted parent. These characteristics of hybrid wood ants may significantly increase their chances of persisting with rapid climate change. This study highlights the beneficial outcomes of hybridisation, which can promote adaptation to climate change.



Predicting the impacts of future climate change on Arctic Charr populations in the Canadian north using genomic offset analyses

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MEETING ROOM 120+121, August 21, 2025, 2:00 PM - 5:00 PM

Climate change represents one of the greatest threats to biodiversity globally, and northern Canadian freshwater ecosystems are predicted to be disproportionately impacted. Arctic Charr (*Salvelinus alpinus*) are an ecologically, economically, and culturally important species throughout northern Canada, with anadromous populations a particularly important resource for northern communities. Anadromous Charr undertake seasonal migrations to feed in the productive marine environment, with the timing of their outmigration influenced by factors such as ice break up and sea surface temperature. Nonetheless, despite their importance and vulnerable habitat, how Arctic Charr will respond to a changing climate remains a largely unanswered question. To understand how local adaptation may contribute to the resilience or vulnerability of different Charr populations to climate change, recent work has modeled the 'genomic offset' (the mismatch between current adaptive genomic variation and that required under future environmental conditions) of populations across coastal rivers in Labrador, Canada, and predicted a loss of southern anadromous populations. However, question remains as to if genomic offset models that include environmental

variables explicitly characterizing the marine habitat will generate predictions that align with those from previous models based on air temperature and precipitation data. Additionally, it remains to be determined how Charr populations across the broader landscape of the Canadian north may respond to climate change, as well as the extent to which predictions made using genomic data will align with those generated via other methods. Here, we use a geographically extensive low-coverage whole genome resequencing dataset to build on previous work and conduct genomic offset analyses on Charr populations in northern Canada, using predictor variables representing environmental conditions experienced by Charr in both marine and freshwater habitats. We also compare these offset predictions to species distribution models of future charr occurrence patterns in the Canadian north. The results generated from this work will form a better understanding of the various factors that may contribute to Charr resilience or vulnerability in the future and how different datasets can be integrated to form stronger, more nuanced predictions, and ultimately will prove valuable for use in assessment and management purposes.



The evolution of seasonal gene expression in forest trees

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MEETING ROOM 120+121, August 21, 2025, 2:00 PM - 5:00 PM

Background: The life histories of organisms are tightly linked to seasonality. Phenology, the temporal coordination of biological activities, such as leaf flushing and flowering in plants, is governed by gene expression dynamics in response to seasonal environments. However, despite its significant role in the adaptation to the seasonal environment, the evolutionary mechanisms of seasonal gene expression remain unclear.

Materials and methods: We compared genome-wide expression dynamics under natural seasonal conditions (molecular phenology) in four dominant evergreen Fagaceae species in Asia (*Quercus glauca*, *Q. acuta*, *Lithocarpus edulis*, and *L. glaber*), using leaf and bud tissues over two seasonal cycles. To quantify the transcript abundance in these non-model tree species, we first assembled high-quality reference genomes for *Q. glauca* and *L. edulis* and identified 11749 single-copy orthologous genes. To assess the similarity of gene expression profiles across tissues, species, and seasons, we performed hierarchical clustering of seasonal transcriptome profiles. Additionally, we applied a non-parametric rhythm detection algorithm RAIN to identify genes exhibiting seasonal expression dynamics and quantified their periods and peak months. Based on these quantifications, we evaluated the divergence in seasonal gene expression patterns across species by calculating the peak month

differences between species pairs. Furthermore, we investigated sequence divergence using the evolutionary rate (dN/dS) and related it to the divergence of seasonal expression patterns.

Results: Seasonal transcriptomic profiling revealed highly conserved gene expression across species in winter, particularly in buds, when temperatures fall below ~10°C. Rhythmic gene expression was detected more frequently in buds (51.9%) than leaves (40.6%), with the majority of rhythmic genes (78.4–92.0%) displaying annual periodicity, while only a small fraction (1.2–11.9%) showed half-annual cycles. The seasonal peaks of rhythmic genes were highly synchronized across species in winter but diverged during the growing season, aligning with key phenological events such as leaf flushing and flowering. Genes with conserved seasonal peaks exhibited slower protein coding-sequence evolutionary rates, suggesting weak selective pressures maintaining seasonal gene expression, while the evolutionary rates were similarly slow regardless of whether genes were expressed in winter or the growing season.

Conclusions: These findings suggest that a shared "molecular calendar" emerges in winter, constraining gene expression evolution and potentially limiting temporal niche partitioning and species divergence in seasonal environments.





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S31 - Habitat-forming species and global change: a multidisciplinary perspective on their evolution and adaptive potential to improve their conservation



‘Green tide’ on the Korean coast: the major green tide forming species, biomass, and ecological impacts on benthic invertebrate ecosystems

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POSTER SESSION 1 - P01.324, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: There has been a sharp increase in the occurrences of ‘green tide’ worldwide – the rapid proliferation of green macroalgae, the genus *Ulva* (Chlorophyta) – which can be attributed to accelerating climate change and anthropogenic influences. This nuisance phenomenon has detrimental impacts on coastal ecosystems by significantly reducing local biodiversity and causing severe economic damage. In South Korea, while green macroalgal blooms have persisted along the coastlines of Jeju Island (which is located off the southernmost region of the Korean Peninsula) since the early 2000s, they have also been sporadically observed on the southern coasts of the mainland. However, which *Ulva* species primarily account for the local green tide incidences, biomass, spatio-temporal variation in *Ulva* community structure, and also ecological impacts on the community ecosystems remain largely unexplored.

Materials and methods: We examined the *Ulva* community structure on Jeju Island and the South Sea at a seasonal basis by conducting comprehensive phylogenetic analysis on 966 specimens from 46 sites using chloroplast barcode *tufA* (chloroplast elongation factor Tu) gene as a molecular marker. Nuclear 5S rDNA (5S ribosomal DNA) was further analyzed for 105 *Ulva* specimens to identify the species within a LPP (*Ulva linza*-*prolifera*-*procera*) clade. By using environmental DNA (eDNA)-based metabarcoding, we investigated the effects of green algal mats on the invertebrate diversity in the benthic ecosystems.

Results: A total of 9 *Ulva* species were found on Jeju Island, with *Ulva ohnoi* and *Ulva pertusa* (= *Ulva australis*) being the most predominant and green tide causing species throughout the year. By comparison, in the South Sea, *U. pertusa* and *Ulva linza* were the most dominant species responsible for the local green tides. Considerable spatio-temporal variation in the *Ulva* community structure was observed between the island and mainland habitat environments. Moreover, we found that phylum Mollusca was predominant in high green tide benthic environments, whereas phyla Cnidaria and Mollusca were dominant in low green tide ecosystems.

Conclusions: The findings of this study will provide crucial genetic/taxonomic information on *Ulva* species in Korean coastal areas, which will aid in the development of management strategies to mitigate the negative impacts of green tides. Further research needs to determine environmental and ecological factors accounting for the local green tides in Korean waters.

Acknowledgements: This work was supported by Ministry of Oceans and Fisheries of Korea (Development of Adaptation Strategies for Marine Environments in Response to Subtropicalization: Scenarios, Solutions, and Utilization; RS-2025-02304432) and also through Agriculture and Food Convergence Technologies Program for Research Manpower Development Project, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (grant number RS-2024-00400922).



Hidden Diversity: Genomic Evidence of Cryptic Species and Habitat Specialization in the Brooding Coral *Seriatopora hystrix* Along the Great Barrier Reef

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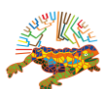
POSTER SESSION 1 - **P01.322**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Identifying differences in environmental adaptations among and within species is fundamental to understanding ecological and evolutionary processes. Corals are habitat-forming organisms integral to reef ecosystem functioning and present excellent systems to study such adaptations. Genomic studies increasingly reveal that many coral taxa are comprised of morphologically indistinguishable yet genetically distinct species occurring across diverse habitats in sympatry. There is also evidence that these cryptic coral species may be specialised to distinct environmental niches, suggesting potential for differential adaptive responses to changing ocean conditions. However, investigations of cryptic coral diversity have predominantly focused on well-studied broadcast spawning genera such as *Acropora*, with comparatively little attention given to brooding corals despite greater opportunities for local adaptation in the context of limited dispersal and gene flow.

This study aims to investigate the genomic basis of cryptic diversity and local adaptation in brooding corals across environmental gradients of the Great Barrier Reef (GBR). To do this, we sampled corals within the species complex *Seriatopora hystrix* along an expansive latitudinal gradient of the GBR. We employed low-coverage whole-genome

sequencing to assess genome-wide variation and divergence within and among putative cryptic species. Our analyses of over 100,000 single-nucleotide polymorphisms revealed at least three distinct genetic lineages, with two co-occurring in sympatry at several sampling locations. We further identified genetically distinct populations associated with specific depths, habitat types, and reef locations, suggesting fine-scale local adaptation.

Ongoing analyses are focused on identifying candidate genes under environmental selection using genotype-environment association analyses and genome scans. We also aim to examine micro-morphological skeletal characteristics and symbiont community composition across the three distinct taxa to determine the functional basis of their divergence. As coral reefs face increasing threats from climate change, our results highlight the importance of recognizing evolutionarily distinct and differentially adapted coral lineages within nominal species. Our findings have important implications for protected area design and restoration initiatives. Conservation efforts should consider preserving and harnessing this cryptic diversity to maintain adaptive potential and resilience in coral reef ecosystems.



Maximising adaptive capacity and climate resilience in structural rainforest trees: a versatile population genetics workflow for both restoration and conservation outcomes

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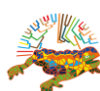
POSTER SESSION 1 - P01.325, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Ensuring both conservation collections and newly restored plantings are genetically representative and resilient to global environmental change is needed for establishing persistent, self-sustaining populations, particularly for long-lived, habitat-forming species like rainforest trees. Effective provenancing strategies must therefore capture genetic diversity from across both local and climate-adjusted regions of a species' range to enhance adaptive capacity under future scenarios. While local and climate-adapted provenancing approaches exist, broadly applicable workflows that maximise genetic diversity while addressing practical restoration challenges are lacking.

Developed through the Science Saving Rainforests project - a collaboration between the Big Scrub Rainforest Conservancy, the Botanic Gardens of Sydney, and Firewheel Rainforest Nursery, this study provides a population genetics-based workflow guiding sampling for over 60 subtropical rainforest tree species. These include widespread key-structural species to narrowly distributed threatened taxa. We present a framework to address three central questions:

1. How many source trees are needed to capture representative diversity within a genetic neighbourhood?
2. How many additional trees enhance adaptive potential by integrating diversity from beyond the local gene pool? (i.e. incorporating diversity from an external genetic neighbourhood)
3. Can practical constraints (e.g., geopolitical boundaries, restricted access or limited nursery space) be reconciled to achieve optimal genetic outcomes?

Using a novel optimisation approach incorporating a down-projected site frequency spectrum (implemented via the OptGenMix R package), we demonstrate how sampling strategies can be tailored to maximise common allele representation across genetic neighbourhoods while remaining feasible for practitioners. In all cases, genetically informed workflows outperform conventional practices that rely on narrowly local or limited sources. This work illustrates how integrating population genetics into restoration planning can safeguard both local adaptation and future resilience, offering a scalable model for conservation and restoration of habitat-forming species under climate change.



Intraspecific variability in thermal tolerance shapes future declines in macroalgal forests

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MEETING ROOM 131, August 18, 2025, 3:30 PM - 5:00 PM

In response to anthropogenic climate change, the geographical distributions of many species are shifting. Accurate forecasting of species responses to warming is particularly important in marine ecosystems, where temperature plays a central role in shaping species distribution. Local environmental conditions and biological processes, such as phenotypic plasticity and/or local adaptation, may result in diverse intraspecific responses to thermal stress. However, most predictive distribution models assume that species responses are homogeneous throughout their distributional range, and the heterogeneity of the responses to warming at population scale remain poorly understood. In this study, we aim to predict the vulnerability of a canopy-forming furoid seaweed, *Ericaria crinita* (Duby) Molinari & Guiry, to future warming trends throughout the Mediterranean Sea, while taking into consideration the intraspecific variability in thermal tolerance. First, we experimentally assessed the variability in the thermo-tolerance response of eight populations across different temperature regimes, covering approximately 2000 km longitude.

We then combine linear mixed models, population genetics and environmental sensitivity analyses to decipher the underlying processes. Finally, we integrate the thermal tolerance data with a distribution modelling approach to accurately predict the species' vulnerability under the RCP 8.5 scenario for year 2050 across its entire geographical distribution. Our results show a clear intraspecific variability in the temperature tolerance thresholds among populations, likely driven by local adaptation. While populations from the coldest zones of the species' distribution show the lowest temperature tolerance thresholds, predictive modelling shows that future warming will lead to similar vulnerability among populations, regardless of their geographical distribution. Our results indicate, however, that disregarding the intraspecific variability in temperature tolerance may result in an under- or overestimation of species vulnerability to future warming at the regional scale, thus highlighting the importance of considering fine-scale data to obtain realistic predictions of species responses to climate changes.





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S32 - Letting go: reductive evolution across the tree of life



Illuminating the mystery of thylacine extinction: a role for relaxed selection and gene loss

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¹IISER Bhopal

POSTER SESSION 2 - **P02.265**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Gene loss shapes lineage-specific traits but is often overlooked in species survival. We investigate the role of ancestral gene loss using the extinction icon—thylacine (*Thylacinus cynocephalus*). While studies of neutral genetic variation indicate a population decline before extinction, the impact of thylacine-specific ancestral gene losses remains unexplored. The knowledge of gene losses in extinct species could provide valuable insights into the causes of species extinction, refine de-extinction strategies, and offer clues about evolutionary processes, such as trait evolvability.

Materials and methods: We leverage paleogenomic data of thylacine to examine gene presence and absence patterns compared with the Tasmanian devil (*Sarcophilus harrisii*). We began with a genome-wide screen for gene losses in the thylacine, corroborating these loss events with short-read Illumina sequencing data. Additionally, we also reconstructed the gene loss history using genomes from other

marsupial species and assessed relaxed selection signatures.

Results: We discovered ancestral (an interval of 13-1 Ma) loss of *SAMD9L*, *HSD17B13*, *CUZD1* and *VWA7* due to multiple gene-inactivating mutations, corroborated by short-read sequencing. The timing of gene loss mirrors the thylacine's shift toward hypercarnivory and increased body size. Our genome-wide analysis reveals olfactory receptor loss and relaxed selection, aligning with reduced olfactory lobes in the thylacine, indicating olfaction is not its primary hunting sense.

Conclusion: By integrating palaeogenomic data with comparative genomics, our study sheds light on ancestral gene losses and their impact on species fitness while providing a foundation for selecting candidate species and genes for thylacine de-extinction. Our approach can be extended to other extinct/endangered species, helping to identify genetic factors for de-extinction efforts.



Increasing mutation rates and population sizes both lead to a reduction in the genome, but not in the same genome compartments

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POSTER SESSION 2 - P02.264, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Genome reduction is commonly observed in bacteria with very different life traits, including endosymbiotic bacteria and several marine bacteria, raising the question of its evolutionary origin. However, the evolutionary determinants of reductive evolution are difficult to assess, mainly due to the many confounding factors associated with the diversity of species with reduced genomes. Computational models avoid this difficulty by allowing the same (artificial) species to evolve in very different evolutionary contexts in order to rigorously test hypotheses.

Aevol is a computational platform designed to study the evolution of genome architecture and complexity. In Aevol, individuals are represented by a genome with a realistic structure, allowing to analyze its complexity in terms of genome size, gene number, or coding fraction. This genome is decoded into a mathematical function representing the phenotype. Finally, the organism's fitness is calculated by comparing its phenotype with the optimal phenotype given the environment. Aevol can be used to test hypotheses on the evolution of complexity and the conditions leading to a reductive evolution. Importantly, the genotypic, phenotypic, and environmental complexities are only loosely coupled: complex genotypes can encode simple phenotypes, and complex organisms can thrive in simple environments.

In a first series of experiments, we used Aevol to evolve populations in a simple environment. This enabled us to show that complexity obeys a "complexity ratchet" that is very difficult to overcome, even in conditions where losing complexity would be favorable. Specifically, we showed that complexity loss occurs only in case of an elevated requirement for mutational robustness and that this can be triggered by an elevated mutation rate [1].

Going further on this idea, we tested whether an increase in population size or mutation rate could cause genome reduction. To this aim, we used pre-evolved individuals that possessed

both a complex genotype and a complex phenotype and transferred them into new conditions characterized by an elevated mutation rate or population size. We observed that both conditions led to genome reduction but resulted in very different genome structures [2]. Under increased population sizes, genomes lose a significant fraction of non-coding sequences but maintain or increase their coding size, resulting in densely packed genomes (akin to streamlined marine bacteria genomes). By contrast, under an elevated mutation rate, genomes lose both coding and non-coding sequences (akin to endosymbiotic bacteria genomes). Hence, only an elevated mutation rate leads to a simultaneous simplification of both the genotype and the phenotype. Further analyses suggest that genome size and coding density are determined by the interplay between selection for phenotypic adaptation and selection for robustness. An elevated population size increases the strength of both selective forces. This leads to maintaining or increasing the coding compartment (higher selection for phenotypic adaptation), but simultaneously it strongly decreases the non-coding compartment (higher selection for robustness). On the contrary, increasing the mutation rate leads to a strong loss of robustness, hence engaging a mechanism of error threshold that results in a loss of both coding and non-coding genomic elements.

[1] Liard, V., Parsons, DP., Rouzaud-Cornabas, J., and Beslon, G. (2020). The complexity ratchet: Stronger than selection, stronger than evolvability, weaker than robustness. *Artificial life*, 26(1), 38-57.

[2] Luiselli, J., Rouzaud-Cornabas, J., Lartillot, N., and Beslon, G. (2024). Genome streamlining: effect of mutation rate and population size on genome size reduction. *Genome Biology and Evolution*, 16(12), evae250.



Molecular basis of convergent evolution: parallel reduction of the sporophyte phase in mosses

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POSTER SESSION 2 - **P02.268**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Whether evolutionary trajectories leading to a particular function/morphology are predictable is a fundamental question of evolutionary biology. Yet, there is little consensus on this issue and experimental evidence is contentious. The phenomenon of convergent evolution, the repeated evolution of traits in independent lineages, provides ideal replicates to test for constraints on the trajectory of the evolutionary processes.

To study this question, we investigate the genetic bases of parallel morphological evolution in a closely related group of moss species, the family Funariaceae. Fitting the classical example of parallel evolution, the reduced sporophyte phenotype has evolved multiple times independently in the Funariid mosses. Here we studied two species which represent the end points of sporophyte complexity within the family. *Funaria hygrometrica* has an elongated seta and specialized structures for controlled spore release, while *Physcomitrella patens* exhibits a reduced sporophyte phenotype, characterized by a short seta and a simple spherical capsule. By comparing gene

expression patterns throughout sporophyte development with whole genome sequence data, we found that both heterochronic changes in the expression of highly conserved homologous transcription factors and species-specific gene gain and loss contribute to the evolution of divergent sporophyte morphologies. Interestingly, heterochronic expression changes are mainly associated with size modulation, whereas gene gain and loss underlie the origin or loss of specific morphological characters. Furthermore, comparative genomic analyses suggest that morphological diversification is associated with significant changes in genome size and transposable element movement, contributing to regulatory rewiring and gene set variation among species.

Taken together, our analyses suggest that both the rewiring of existing regulatory networks and the addition/loss of non-conserved genes to regulatory cascades, have contributed to the evolution of a reduced sporophyte phenotype in Funariaceae.



Neural and Molecular Signatures of Olfactory Specialization in Ant Social Parasites

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POSTER SESSION 2 - **P02.263**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Chemical communication is central to the collective behaviour of ants and is primarily mediated by their olfactory system. This system depends on odorant receptors (ORs) expressed in olfactory sensory neurons, whose axons project to the antennal lobe, where they form spherical neuropil structures known as glomeruli. In *Drosophila*, each OR is typically expressed in a single neuron type, which targets a single glomerulus, establishing a one-to-one correspondence between receptor, neuron, and glomerulus. In ants, the OR repertoire has undergone a substantial expansion, accompanied by an increase in the number of glomeruli.

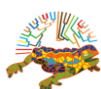
Obligate slave making ants are social parasites that depend on host colonies for all worker tasks, including brood care and foraging. These parasites capture the host worker's brood during raids, and the emerging workers integrate into the parasite colony, where they perform all essential colony functions. Four independently evolved slave making species have convergently lost approximately one-third of their ORs, specifically those that are differentially expressed in the antennae of brood carers and foragers in their hosts. This pattern raises several questions about the consequences of OR loss on neural architecture and sensory function in slave-making ants. Does the reduction in ORs lead to a corresponding decrease in glomeruli, potentially affecting olfactory processing? Are OR genes linked to raiding behaviour shared across species, and do they show convergent signatures of selection in independently evolved slave-making ant lineages?

To determine whether glomerular reduction is a consistent feature of slavemaker brain anatomy, we quantify glomeruli in the antennal lobes of slave-making ants and their hosts

using immunostaining and confocal microscopy. We plan to analyze the number of glomeruli in four independent origins of slave-making behaviour in ants: *Temnothorax americanus*, *Temnothorax pilagens*, *Harpagoxenus sublaevis*, and *Myrmoxenus ravouxii*, along with their respective host species.

We aim to increase our sample size, but our initial results demonstrate that the antennal lobes of *Temnothorax americanus*, a slave-making species, harbor 310–340 glomeruli per antennal lobe ($N = 3$), compared to 420–450 in its host species, *T. longispinosus* ($N = 7$). We determine whether glomerular reduction is a consistent feature of slave-maker brain anatomy. These glomeruli count closely reflect the lower number of OR genes in the host in comparison to the slave maker: 308 in *T. americanus* (slave-maker) and 423 in *T. longispinosus* (host).

To identify ORs involved in raiding behaviour in slave-making ants and defensive behaviour in their hosts, we conducted slave raids in controlled laboratory conditions, allowing *T. americanus* colonies to raid nests of *T. longispinosus*. We sampled workers from both species that participated in the raids, as well as non-participating individuals. RNA was extracted from the antennae and brains of these workers, followed by RNA sequencing. We plan to present the results of our analysis identifying differentially expressed ORs associated with raiding and defensive behaviours and to test whether these genes exhibit parallel signatures of selection in different slave-making and host systems. This work aims to clarify the evolutionary dynamics of the olfactory system in the context of specialized social parasitism in ants.



Tracing genome reduction among prokaryotic lineages to reveal shared patterns and drivers of gene loss

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POSTER SESSION 2 - **P02.266**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Gene loss is a common evolutionary phenomenon in diverse prokaryotic lineages. This is especially well documented for parasitic and mutualistic (endo)symbionts, but it also occurs in abundant marine oligotrophs (e.g., *Prochlorococcus* and *Pelagibacter*) and certain extremophiles. Depending on the lifestyle of the lineage, different drivers of these genomic changes have been proposed. In endosymbionts, genes required only needed for survival outside of the host cytoplasm experience relaxed selection pressure, allowing for their loss. Additionally, population bottlenecks can result in gene loss through genetic drift – especially in vertically transmitted endosymbionts. In oligotrophs and extremophiles, gene loss is often considered beneficial to reduce the metabolic cost of maintaining a larger genome in resource-limited or stressful environments. For oligotrophs, it has additionally been suggested that gene loss is linked to changes in effective population size and an increase in mutation rate. While studies of specific lineages have been fruitful in outlining these different drivers of gene loss, a comprehensive overview across the trees of

Bacteria and Archaea is missing. Moreover, recent expansions of the known diversity of prokaryotes may have uncovered novel lineages that have undergone gene loss. In this project, we aim to identify the bacterial and archaeal lineages that have undergone substantial gene loss based on variation in gene counts and content by making use of the wealth of currently available sequence data. Lineages of interest will then be analyzed in greater detail by reconstructing species trees. We will analyze these lineages by reconstructing how they lost, gained and transferred genes during evolution using gene tree-species tree reconciliation methods. Changes in mutation rate and selective pressure within these lineages will be studied with estimations of non-synonymous and synonymous substitutions and their ratio (dN/dS). By comparing both the potential drivers of gene loss and the resulting gene content between lineages, we aim to identify both lineage-specific and convergent patterns of genome reduction. These patterns may correlate with lifestyle, providing insight into the generality of gene loss and its evolutionary drivers across microbial life.



Genomic signature of CAM photosynthesis loss

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MEETING ROOM 131, August 21, 2025, 2:00 PM - 3:30 PM

CAM photosynthesis has evolved multiple times across the plant kingdom. While it provides greater water-use efficiency under arid conditions, it's also a costly adaptation. The genomic processes accompanying the loss of CAM once it is no longer advantageous remain largely unexplored. Members of the Montiaceae family express variable amounts of CAM photosynthesis and are broadly described as C3+CAM plants, able to perform CAM in addition of the regular C3 pathway from which they source most of their carbon. In this study, we investigate a plant lineage within this family - the genus *Montiopsis* - which occupies Andean alpine habitats and has secondarily lost the capacity to perform CAM. This

configuration offers a rare window into the evolutionary dynamics of metabolic pathway reversal. Using comparative genomics, we identify a suite of molecular changes in *Montiopsis* compared to its C3+CAM relatives, consistent with ongoing degeneration of the CAM pathway. Several key CAM genes have been lost or pseudogenized and we detected shifts in selective pressure on others, suggesting some of them may be reused in other processes. Together, these findings suggest a multi-phase process of functional decay, highlighting how metabolic flexibility in plants is underpinned by dynamic and reversible genomic architectures.



Unraveling the reductive evolution of bdelloid rotifers, while having adapted to extreme stresses.

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MEETING ROOM 131, August 21, 2025, 2:00 PM - 3:30 PM

Bdelloid rotifers are microscopic animals notorious for having lost males and canonical sexual reproduction. This paradox is often counterbalanced by their remarkable ability to survive extreme genome damage caused by desiccation and ionizing radiation, having colonized a broad range of semi-terrestrial habitats. Our research group, through an ERC-funded project, has been investigating how bdelloid rotifers survive both the challenges of asexuality and the biological toll of extreme stresses. Using an integrated approach combining omics data, molecular biology and cytological analyses, we focused on the model species *Adineta vaga*. We discovered that *A. vaga* undergoes a non-reductional meiosis. While their six homologous chromosomes do pair and recombine, the first meiotic division is abortive, and the homologous chromosomes do not segregate. Strikingly, DNA repair in the germline is delayed until this stage of oogenesis, allowing homologous recombination-based repair of radiation-induced damage. In contrast, DNA repair in somatic cells is rapid and occurs shortly after DNA damage. Our experimental evolution study revealed megabase scale losses of heterozygosity at genome level, confirming the occurrence of meiotic homologous recombination. Unexpectedly, we also detected large-scale duplications and deletions, and the propagation of broken chromosomes across generations, that gets progressively repaired through a novel transgenerational DNA repair mechanism

based on extended homologous recombination repair. Therefore, despite the loss of outcrossing, their simplified meiosis, together with the presence of holocentric chromosomes, has made bdelloid rotifers more resilient to aneuploidy and mis-segregation events. Beyond their simplified parthenogenetic reproductive mode, bdelloid rotifers exhibit other signs of reductive evolution. They are eutelic, with a fixed number of cells and many tissues organized as syncytia, reducing the need for cell division. Their genomes appear compact (around 100Mb in *A. vaga*), with a minimal content of repetitive and transposable elements, and a notable elimination of non-coding regions compared to their sister clade, the monogonont rotifers. Interestingly, bdelloid rotifers have accumulated horizontally transferred genes (HTGs) of non-metazoan origin, particularly in subtelomeric regions. Some of these genes, such as Ligase E, play key roles in their stress tolerance. Bdelloid rotifers are not only innovative at repairing DNA but have also evolved robust protein protection systems, enabling them to resist oxidative damage and efficiently recycle cellular components. Altogether, bdelloid rotifers exemplify how a combination of reductive and innovative evolutionary strategies can enable long-term survival without sex and in the face of extreme environmental challenges.





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S33 - Linking recombination rates and supergene evolution with the genomics of complex traits



Complex supergene system and fine-scale dispersal in a socially polymorphic ant

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POSTER SESSION 3 - **P03.101**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Supergenes play an important role in the evolution of complex phenotypes, locking specific combinations of genes by partially suppressing recombination. Social polymorphism in ants (the presence of single (monogynous) or multiple (polygynous) reproductive queens within colonies) is one of such complex phenotypes. It is associated to divergent individual and colonial traits, some of which linked to dispersal. In ants, the dispersal phase occurs during colony foundation. The association between social polymorphism and colony foundation strategy is a long-standing paradigm in ants evolutionary ecology: monogyne queens found new colonies independently (ICF), while polygyne queens can be readopted or found a new colony with the help of workers (dependent colony foundation, DCF). Queen adoption and DCF (workers are wingless) strongly limit female dispersal. Males hence play a crucial role for maintaining gene flow and avoid inbreeding.

Here we investigated the complex interaction between sociality, colony foundation and dispersal in an ant species, *Myrmecina graminicola*. This species combines a social and a wing polymorphism (presence/absence of wings in queens) which are not strictly associated, contrary to the prevailing paradigm. The two polymorphisms are genetically determined by a supergene characterized by three haplotypes (Mo, Ma and Pa) whose genotypic combination controls the three observed phenotypes: monogynous winged queens (MoMo), monogynous wingless queens (MoMa), polygynous queens (either MoPa or MaPa). Apterism is determined by a ~110 kb insertion which is present in both Ma and Pa haplotypes, and which discriminates Ma from Mo. Mating strategies (intra-nidal mating, assortative mating) should affect male gene flow and female dispersal (by flight or by foot, alone or together) and should leave distinct footprints on

the patterns of genetic variation of the three morphs within and outside the supergene. Specifically, polygynous queens are expected to exhibit greater population viscosity (higher relatedness) and reduced genetic diversity because of limited dispersal.

To test for this hypothesis, we whole-genome sequenced 118 queens from 49 georeferenced colonies (33 MoMo, 5 MoMa, 13 MoPa) sampled within a restricted geographical area (~0.1 ha). We then contrasted the spatial relatedness, consanguinity (measured by runs of homozygosity, ROH) and genetic diversity (measured by the site frequency spectrum, SFS) of the three morphs. Overall intra-nest relatedness in polygynous colonies revealed that most of the queens were sisters, but adoption of unrelated queens occurs in a third of the colonies. Strikingly, genetic and geographic distance were not correlated at this small spatial scale for all the three morphs, suggesting similar dispersal ability and/or significant male gene flow independently of the sociality and the wing polymorphism. Consistently with this finding, but contrary to the expectation in case of restricted dispersal, we found no differences in genome-wide ROH among all queens, implying that the two morphs have similar inbreeding level. However, we detected small but significant variation in the SFS between monogynous apterous vs winged queens (after controlling for sample size), with the former displaying higher diversity and an excess of low frequency variants compared to the latter. We speculate that the insertion, present in all apterous queens and found in diploidy at low frequency, may promote gene flux between Ma and Pa, increasing diversity in monogynous apterous queens compared to winged queens. Ongoing analysis at the edges of the insertion will likely provide new insights about the dynamics of mating strategy among social morphs.



Degeneration and gene expression patterns in the morpho-social supergene in the ant *Myrmecina graminicola*

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POSTER SESSION 3 - P03.104, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Supergenes play an important role in the evolution of complex phenotypes, locking together specific combinations of genes by suppressing recombination between haplotypes. Ultimately, this leads to the degeneration of the non-recombining haplotype, i.e., the haplotype which is rarely (or never) found at the homozygous state. Degeneration occurs through the accumulation of repeated elements and deleterious variants, due to the reduction in the efficiency of purifying selection. Moreover, the accumulation of deleterious variants should lower the expression levels of genes of the non-recombining haplotype.

In this work, we focus on the complex supergene system recently discovered in the ant *Myrmecina graminicola*. This species combines a social polymorphism, i.e., the presence of colonies with single (monogynous) or multiple (polygynous) queens, and a wing polymorphism, i.e., the presence/absence of wings in the queen. The two polymorphisms are genetically determined by a supergene characterized by three haplotypes (Mo, Ma and Pa) whose genotypic combination controls the three observed phenotypes: monogynous winged queens are MoMo, monogynous wingless queens are MoMa, polygynous queens are MoPa or MaPa. Apterism is determined by a ~120 kb insertion which is present in both Ma and Pa haplotypes, and which discriminates Ma from Mo. Importantly, Pa is the derived and non-recombining haplotype, as no PaPa queens have been found until now.

To investigate the consequence of recombination suppression we assembled a new Mo and a new Pa genome by combining long-reads and Hi-C sequencing. Furthermore, we conducted a differential gene expression analysis by comparing RNA-Seq data obtained

from the heads and thorax of 10 monogynous winged (MoMo) and 10 polygynous apterous (MoPa) queens.

Comparing the structures and the annotations between Pa and Mo revealed that the former was ~4 Mb longer and presented a higher concentration of repeated elements than the latter (35% vs 25% respectively). Furthermore, we found 832 genes in Pa including 7 in the inserted region, while only 716 genes were observed in Mo.

We then mapped the RNAseq reads against the Pa haplotype and tested whether (i) genes within the supergene are more differentially expressed between the two morphotypes as compared to the rest of the genome and (ii) the accumulation of deleterious mutation lowers gene expression within the Pa haplotype. After controlling for the effect of body parts, we observed that genes in the supergene tended to be significantly downregulated in polygynous queens, compared to randomly drawn genes in the rest of the genome (Student's t-test p-value < 1e-8). We further obtained a total of 24 differentially expressed genes between monogynous and polygynous queens, out of which 18 were located in the supergene region and more expressed in monogynous individuals, representing a significant enrichment compared to the rest of the genome (Fisher's Exact Test p-value < 0.05). Finally, mapping quality, due to the high divergence between Mo and Pa, can bias differential expression analyses, which we are currently replicating using Mo haplotype as reference. Our study also revealed that a single gene in the "apterism" insertion was expressed. This gene is orthologous to a gene present in chromosome 4, making it a strong candidate for the causal factor determining apterism.



Inversions in the background: Genomic inversions facilitate rapid adaptation by modulating sexual conflict and regulatory interactions in Hawaiian field crickets

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POSTER SESSION 3 - P03.103, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Genomic inversions often play direct roles in adaptive evolution by maintaining beneficial genetic interactions and reducing gene flow between populations, thereby enabling local adaptation. However, we propose a new model in which ancestral, polymorphic inversion exerts background effects on adaptive mutations elsewhere in the genome, accelerating adaptation under extreme selective stress. Using adaptive, X-linked, male-silencing mutations in Hawaiian crickets attacked by eavesdropping parasitoids, we found that these adaptation-accelerating effects arise through epistatic regulatory interactions that modulate sexual conflict. We showed this by constructing a comprehensive pan-genomic graph from multiple de novo cricket population assemblies, we identifying a significant ancestral inversion associated with – but not causally determining – the

silent male phenotype, and revealing sex-biased regulatory interactions between the inversion and the known male-silencing locus using integrated analyses of differential gene expression and gene co-expression networks. Evolutionary modelling under various scenarios of epistasis involving the selected locus and the inversion demonstrated how female-compensatory interactions between genomic inversions and male-beneficial loci influence adaptive rates. Our findings show how that ancestral genomic inversions can act as crucial permissive background by reducing sexual conflict and facilitating rapid phenotypic adaptation in natural populations, providing a novel model of how large genomic structural variants underpins adaptation.



Is genomic compatibility compromised after a bottleneck in Asian elephants?

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POSTER SESSION 3 - P03.105, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Despite extensive efforts to curb biodiversity loss, species decline continues at an alarming rate. Large mammals, such as the Asian elephant (*Elephas maximus*), are particularly vulnerable due to habitat loss and fragmentation, which reduce population sizes and intensify genetic drift. Their long generation times limit adaptive potential, leaving them especially susceptible to extinction.

The Asian elephant, an iconic species, has experienced a dramatic population decline, with over 50% lost in just three generations. As of 2024, four subspecies are recognized by the IUCN, with Bornean elephants recently elevated to subspecies status. This population has undergone the most severe bottleneck and exhibits marked genome erosion, raising concerns about long-term viability and the potential need for genetic intervention.

Genetic rescue is emerging as a key tool in conservation, aimed at enhancing genetic diversity in small, inbred populations. However, successful implementation requires careful assessment of genomic compatibility to avoid outbreeding

depression. Using both long- and short-read sequencing, we investigated structural variants (SVs) among Asian elephant subspecies. Bornean elephants harbor the greatest number of unique SVs, likely as a result of a strong and recent population bottleneck. Of particular interest is a fixed, complex 16 Mb variant encompassing 315 genes and multiple nested SVs, several of which overlap coding regions. This region contains genes related to hair and skin traits, including the epidermal differentiation complex, and may underlie distinct phenotypic features of the Bornean subspecies. The architecture of this SV suggests it may function as a supergene, potentially maintained by suppressed recombination, although its exact structure—possibly an inversion—remains to be fully resolved.

We discuss the evolutionary origin and selection history of this SV, and the potential consequences of genetic rescue strategies that could dilute its frequency, highlighting the need for genomically informed conservation decisions.



Palms, a new study system for sex chromosome evolution in plants

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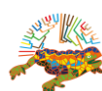
POSTER SESSION 3 - P03.099, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Sex chromosomes evolved many times independently in the tree of life, and in particular in plants, due to a large number of independent transitions towards separate sexes. While all sex chromosomes are characterized by a non-recombining region that is subject to the same processes in all species, recent advances have shown that there is a wide variety in size, divergence, degeneration, and gene content, in particular in plants.

Materials and methods: We here use the palm family, which presents several transitions to dioecy (separate sexes), to study the dynamics of sex chromosome evolution. We use a comparative genomics approach based on exome sequencing allowing for comparisons of genes at an intermediate evolutionary scale in non-model organisms. Sex chromosomes and sex-linked genes are detected using a probabilistic method based on population genetics. Using, the genome of the model dioecious palm species (*Phoenix dactylifera*, date palm) as a reference, we then characterize syntenic non-recombining regions, their level of divergence and gene loss.

Results: We identified XY chromosomes in 3 different species pertaining to 2 dioecious groups that both evolved dioecy independently from *P. dactylifera*. *Kerriodoxa elegans* evolved a non-recombining region with remarkable similarity to the previously described region in the date palm, a case of convergent evolution. *Trachycarpus fortunei* and *Chamaerops humilis*, two closely related species, share a sex chromosome but with largely different non-recombining regions. All non-recombining regions are rather small, only weakly divergent and do not seem degenerated, in contrast to what has been observed in some other plants.

Conclusions: Analysis of the functions of genes in these non-recombining regions reveals homology with genes related to flower unisexualization in palms, particularly those associated with hormonal regulation. Whether the frequent sexual system changes in this family limit the divergence of the sex chromosomes, or whether this is caused by other mechanisms (e.g. turnovers, selection) is currently not known, but further study of the palms could shed more light on the evolution of sex and sex chromosomes.



Positive and negative selection on the supergene controlling male mating strategies in Ruff

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POSTER SESSION 3 - **P03.107**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The ruff (*Calidris pugnax*), a Palearctic wading bird species, is characterized by three different male morphs that differ significantly in behavior, plumage color and body size. This fascinating variation in male morphs is controlled by a polymorphism involving a 4.3Mb inversion on chromosome 11, which has a dominant effect on phenotypes but is recessive lethal. Here, we leverage a newly reconstructed haplotype of the female-mimicking male Ruff morph to conduct a selection scan to uncover the selection signatures on each of the ~100

genes located within the inversion. Furthermore, the recent addition of the closely related Buff-breasted Sandpiper (*Calidris subruficollis*) to our genomic dataset allowed inference of selection signatures that was previously unattainable. Our results provide deep insights into the candidate genes responsible for male mating behaviors and plumage variation in Ruff, as well as enhance our understanding of how positive and negative selection acting on structural variants reflects on phenotypic diversity in natural populations.



Species delimitation for a male-only clonal lineage of ants

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POSTER SESSION 3 - **P03.100**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Messor ibericus exhibits a unique reproductive strategy in which queens must clone males of another species, *Messor structor*, to produce the worker caste. Thus, in addition to mating with males of their own species, *M. ibericus* queens also reproduce with *M. structor* males, which they subsequently clone. Consequently, *M. structor* males exist in two forms: "wild" males found in *M. structor* colonies and "clonal" males residing within *M. ibericus* nests. These two types of males are genetically and morphologically distinct. To determine whether clonal males should be considered a lineage of *M. structor* or a distinct species, we conducted several analyses (calculation of the F_{st} fixation index, species delimitation, population structure analyses etc).

While empirical data indicate that clonal males rarely mate with wild-type *M. structor* females, species delimitation and structure analyses show no significant separation between the two groups. Their intertwined origin, low F_{st} , low genetic divergence, and high historical gene flow all support the conclusion that clonal males belong to *M. structor*, despite their morphological differentiation. Their low current hybridization rate with wild-type females may be explained by reduced mating opportunities due to their recent "domesticated" condition within *M. ibericus* nests. Low genetic differentiation but high morphological difference compared to wild counterparts echoes typical cases of domesticated species, which suggests that clonal males may be considered as a domesticated lineage of *M. structor*.



Convergent evolution of inversion-based supergenes in neotropical lepidoptera

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MEETING ROOM 120+121, August 19, 2025, 2:00 PM - 4:00 PM

Background: Inversions suppress recombination and can lead to the formation of supergenes in which multiple, often functionally related loci are tightly linked. In the neotropical butterfly *Heliconius numata*, a candidate supergene formed from multiple inversions around the highly conserved cortex/ivory locus controls wing colour patterning. *Heliconius numata* flies alongside other aposematic lepidoptera, forming “mimicry rings” whose distantly related members display bright convergent warning colours to advertise their unpalatability. We previously demonstrated that genetic parallelism at the cortex/ivory locus controls this convergent colour patterning in 7 species across 120 million years of evolution. Unusually, *H. numata* is highly polymorphic within single localities – a result of its distinctive supergene architecture. Polymorphism of this kind is uncommon but is also seen in two near identically patterned comimics of *H. numata*: *Eresia pelonia*, where the polymorphism is female limited; and *Chetone histrio*, a day flying moth.

Materials and methods: We sequenced the genomes of >160 individuals of *C. histrio* and *E. pelonia*. Using de-novo genome

assemblies, genome scans, GWAS and comparative genomics, we investigate the genetic architecture of the polymorphism in these two ecologically distinct, distantly related comimics of *H. numata*.

Results: Inversions around cortex/ivory have evolved independently in these 3 highly polymorphic species, leading to the parallel emergence of colour patterning supergenes. As in *H. numata*, distinct genetic elements within the candidate supergene appear to control different aspects of colour patterning. Surprisingly, we do not find any male *E. pelonia* homozygous for the inversion allele, suggesting a potential lethal effect. This is reflected in the distorted sex ratio of wild populations.

Conclusions: We find repeated use of not only the same locus, but the same distinctive genetic architecture between convergent species that diverged ~120Mya ago. Whilst local polymorphism is often associated with chromosomal inversions, we uncover for the first time the independent emergence of candidate supergenes producing convergent phenotypes across deep evolutionary time.



Genomic diversity and evolution in the *Anopheles gambiae* complex: the extreme specialisation of *Anopheles bwambiae* to thermal springs in Uganda

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MEETING ROOM 120+121, August 19, 2025, 2:00 PM - 4:00 PM

The closely related and partially inter-fertile mosquito species of the *Anopheles gambiae* complex (AGC) include the major vectors of human malaria in Africa. While these have attracted much attention, little is known about minor vectors like *An. bwambiae*. This species is one of the most enigmatic species in the AGC, highly specialized to the thermal springs in Uganda, in close vicinity with other major vectors (e.g., *An. gambiae*). Previously reported potential introgression between them raised questions about how such species could remain adapted despite the homogenising effects of gene flow. Here we investigated the genomic diversity and evolutionary history of *An. bwambiae*, together with its extreme adaptations to larval habitats in the thermal springs of Uganda. We first de novo assembled the reference genome for this species and compared it to those from other species of the AGC. Then, using whole genome sequencing, bioinformatics, population genomics, and phylogenomics approaches, we investigated the genetic diversity, evolutionary history, and adaptation of *An. bwambiae* (n=42) compared to *An. gambiae* (n=25). Analyses of 13.5 million SNPs across the genomes of the two species revealed

major genomic heterogeneity including a new inversion system on the X chromosome of *An. bwambiae*. Genetic structure confirmed that the two species are well distinct, with no evidence of recent gene flow. Nevertheless, historical introgression was detected not only with *An. gambiae*, but also with other species of the AGC. It involved large chromosomal inversions (e.g., 3La), but not only. In contrast to *An. gambiae*, *An. bwambiae* displayed low diversity, strong inbreeding, and autozygosity, indicative of a very small effective population. Meanwhile, while genome scans revealed strong positive selection on known insecticide resistance loci in *An. gambiae*, no such signal was identified in *An. bwambiae*. Nevertheless selection signals were detected in other parts of the genome of *An. bwambiae*. How this species persisted and adapted despite its small effective population size and strong inbreeding remains a paradox likely involving a balance between historical introgression, chromosomal inversions, and purging of deleterious mutations. This study sheds light on the genetic makeup of one of the most enigmatic members of the AGC, revealing its extreme specialisation and further highlighting the adaptability of these species.



Small Queens, Big Questions: Investigating the Interaction between Two Supergenes in the Ant *Formica cinerea*

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MEETING ROOM 120+121, August 19, 2025, 2:00 PM - 4:00 PM

Supergenes, clusters of tightly linked alleles, have been associated with complex phenotypes across a wide range of eukaryotes. In ants, several supergenes have recently been identified and linked to variation in colony queen number, and are often referred to as “social supergenes.” One of the best-studied examples is the supergene system in the genus *Formica*, where the social supergene is widespread and spans most of chromosome 3. In most *Formica* species where the structure and function of this supergene have been described, two haplotypes are found: M and P. Despite a few exceptions, single-queen (monogyne) colonies exclusively carry the M haplotype, whereas individuals from multi-queen (polygyne) colonies carry at least one copy of the P haplotype.

In the European species *F. cinerea*, we recently discovered a second supergene located on chromosome 9, with two alternative haplotypes: 9a and 9r. This supergene is associated with reduced body size in alates: queens carrying at least one 9r haplotype are up to 20% smaller than those without it. Similar reductions are observed in males. This species also exhibits remarkable genotypic diversity at the social supergene on chromosome 3, with four haplotypes (MA, MD, P1, and P2) and ten distinct genotypes identified. One of the polygyne-associated haplotypes, P2, is in strong linkage disequilibrium with the 9r haplotype. Small queens (microgyne) and small males are found exclusively in polygyne colonies.

Given the extreme complexity of this system, several key questions remain. How are the

P2 haplotype on chromosome 3 and the 9r haplotype on chromosome 9 linked? Our findings show complete independent assortment of these chromosomes in eggs laid by heterozygous P1P2–9a9r queens. This suggests that the observed linkage disequilibrium is not due to physical linkage, but rather to the lethality of certain haplotype combinations. Indeed, mismatched combinations (e.g., P2 with 9a and 9r without P2) are never observed in males, suggesting mismatched haploid eggs likely fail to develop.

A second key question concerns the maintenance of this complex two-supergene system. Across multiple years of sampling, we have never found newly mated queens carrying P haplotypes, suggesting that only unmated queens from monogyne colonies participate in mating flights. This aligns with the observation that winged large queens from monogyne colonies have larger wings and likely higher dispersal ability than both large and small queens from polygyne colonies. Despite these divergent dispersal strategies, assortative mating appears incomplete, and gene flow still occurs between different queen morphs. Preliminary results also suggest that transmission of maternal haplotypes may be at least partially influenced by the genotype of the mate, although further analyses are needed to confirm this pattern. Together, these findings highlight how complex genetic architectures, such as interacting supergenes, can shape and constrain reproductive strategies and social organization in ant populations.



Supergene-associated variation in queen size and number in the ant *Temnothorax longispinosus*

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MEETING ROOM 120+121, August 19, 2025, 2:00 PM - 4:00 PM

Ants exhibit extensive variation in colony social organization, particularly in queen number and reproductive strategies—a variation traditionally attributed to phenotypic plasticity. However, in several independent ant lineages, supergenes—non-recombining genomic regions maintaining co-adapted gene complexes—have been shown to control alternative social phenotypes. Such genomic architectures can facilitate the evolution of complex traits, including social polymorphisms.

In the acorn ant *Temnothorax longispinosus*, colony queen number varies and is associated with ecological factors such as nest site, food availability, climate, and the presence of social parasites. Moreover, three queen morphs differing in reproductive behavior have been described. Until now, this social and morphological variation was thought to be environmentally induced. Here, we demonstrate that it is genetically determined and associated with at least one supergene.

We collected single-queen and multiple-queen colonies of *T. longispinosus* from ten populations, with a primary focus on a population in upstate New York, USA. Queen morphological traits and ovary development were measured to assess associations between social form and reproductive status. We obtained a chromosome-level assembly for *T. longispinosus* and performed whole-genome resequencing of queens from each of the three morphs. This identified a large 14 Mb region on chromosome 6 with two non-recombining haplotypes M and P associated with queen number and body size.

Queens homozygous for haplotype M were large and predominantly found in single-queen colonies. Heterozygous (MP) queens were mainly large and occurred mostly in multiple-queen colonies. In contrast, queens homozygous for the P haplotype were significantly smaller, with their thorax length 11% smaller, and were primarily found in multiple-queen colonies. Interestingly, large individuals were also present among PP queens, and a few inconsistencies were observed, including single-queen PP or MP queens and a MM queen in a multiple-queen colony. We are currently investigating the genomic basis of the third morph, which is characterized by a disproportionately large head and relatively small thorax.

On chromosome 7, we detected a second 2 Mb region with two non-recombining haplotypes, though without a clear association with any phenotypic trait. Unlike the region on chromosome 6, which appears to conform to Hardy-Weinberg expectations, no individuals homozygous for one allele were found on chromosome 7, despite a high heterozygote frequency of 61%.

Our results provide first evidence for the presence of supergenes in *T. longispinosus*. Ongoing work aims to explore the interaction between the two supergene regions and their role in shaping queen morphs and social organization. In addition, we are analyzing supergene frequencies across the species' range, having found that multiple-queen colonies are more common in northern populations. By comparing current data with historical collections from several decades ago, we aim to assess whether climate change has already led to shifts in social structure and associated supergene allele frequencies.





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S34 - Mechanisms of adaptation to changing conditions in microorganisms



Can diatoms use nutrient stores to affect temperature responses over several generations?

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POSTER SESSION 3 - **P03.125**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Diatoms are a diverse group of unicellular phytoplankton that form the basis of all marine food webs and contribute critically to global primary production. Diatoms, like all organisms, grow and function optimally in a given temperature range. Exposures to temperature outside this range can result in suboptimal performance, or at extreme temperatures, cell death. Additionally, biological rate processes—including ecologically important traits such as the growth rate—are inherently temperature dependent.

The ability of phytoplankton to respond to thermal variability through reversible phenotypic plasticity (acclimation) is crucial for their ecological success and survival. Thermal acclimation is not instantaneous, but is instead gradual, lagging behind the thermal shift and spanning a timescale of many generations. This gradual plasticity may help buffer against trade-offs associated with thermal acclimation, especially since complete thermal acclimation to a given temperature can be maladaptive under conditions where thermal shifts occur rapidly and abruptly. In phytoplankton, thermal acclimation can also affect performance across subsequent thermal shifts. Interestingly, acclimation to cold temperatures has been shown to improve growth at very high temperatures, potentially even more than acclimation to warmer temperatures. This surprising resilience may be linked to altered resource uptake and assimilation during cold

acclimation, though this has not been tested empirically.

Using the model diatom *Thalassiosira weissflogii* to address this gap, we investigated whether altered performance following temperature shifts can be explained by a 'nutrient storage effect,' where diatoms that generate larger internal nutrient stores during thermal acclimation are able to perform better across a range of thermal shifts. By manipulating temperature and nitrate conditions across two genotypes, we find that genotypes differ in how thermal acclimation influences their performance under subsequent thermal shifts. Under nitrate replete conditions, acclimation to higher temperatures improves subsequent thermal performance for one genotype, while hindering performance in another. Under nitrate limitation, subsequent thermal performance is largely hindered across genotypes, with thermal acclimation further reducing growth rates in one genotype. However, intracellular nutrient stores alone do not explain these observed patterns in thermal plastic responses across these genotypes, potentially pointing towards the role of other factors—like heat-shock proteins—that could improve performance or mitigate thermal damage following thermal shifts.

These findings contribute to a more nuanced understanding of how thermal acclimation affects subsequent performance during thermal shifts.



Does the lipid metabolic toolbox constrain the evolution of cellular biological processes?

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POSTER SESSION 3 - P03.118, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

We aim to understand how membrane lipid composition constrains the evolution of cell biological processes. To approach this question, our project capitalizes on naturally occurring divergence in physiology and lipid metabolism between two related fission yeasts, *S. pombe* and *S. japonicus*. The two species exhibit profoundly different membrane glycerophospholipid (GPL) compositions. Unlike *S. pombe*, where the bulk of membranes are made of symmetrical GPLs, *S. japonicus* synthesizes abundant asymmetrical GPLs where tails differ in length by 6-8 carbons.

We decided to use laboratory evolution to gain insights into the genetic response to modifications in lipid metabolism. We used a *S. pombe* strain which had been modified by interspecies genes swap to produce asymmetrical GPLs typical of *S. japonicus* in a *S. pombe* strain. This modified strain was allowed to evolve under both benign and stressful conditions, enabling us to investigate adaptations to shifts in membrane lipid composition and their influence on responses to environmental stress.

We conducted a laboratory evolution experiment where 16 populations were grown in parallel both in the absence and presence of acetic acid, a weak organic acid commonly encountered in nature by *S. pombe*. It has been shown that yeast cells undergo lipid remodelling when submitted to acid stress. Adaptation was tracked by monitoring growth rate changes during the experiment (~9 weeks).

Within this timeframe, the growth rate of all populations increased (except for control strains in benign condition) and we were subsequently able to identify the causal mutations. Interestingly, our data show that gene regulation was systematically affected by mutations in several transcription factors in populations of *S. pombe* strains producing asymmetrical GPLs. I will present the phenotypic effects of these mutations and discuss their role in the context of adaptation to lipidome modification.

Our results provide valuable insights into the constraints bearing on the evolution of cellular physiology.



Effect of multiple global change factors on microbial communities in the field

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POSTER SESSION 3 - P03.127, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Anthropogenic activities impose multiple concurrent pressures on soils globally, but responses of soil microbes to multiple global change factors are poorly understood. We applied 10 treatments (warming, surfactant, salinity, nitrogen addition, drought, heavy metal, microplastics, antibiotics, fungicides, and insecticides) individually and in random combinations of 2, 4, 6, 8 and 10 factors to soil samples in the field, and monitor their microbial composition after 2 years of exposure by metagenomic analysis. The increasing numbers of global change factors caused directional changes in the

community composition that could not be predicted from the responses to individual factors. Populations thriving in multiple global change factors were characterized by increasing abundances of rare taxa in control samples, and showed particular functional traits like increasingly reduced genome sizes. Our study indicates that multiple factors impose selective pressures on soil microbes not observed at the individual factor level, and emphasizes the need of studying the effect of concurrent global change treatments.



Emergence and persistence: Structural evolution of early Nitrogenase enzymes

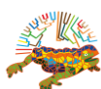
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POSTER SESSION 3 - P03.117, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The evolution of biogeochemical enzymes shaped the biosphere as we know it. Nitrogenases are key ancient enzymes with significant impact on Earth's biosphere, as they are the only known enzymes that convert atmospheric molecular nitrogen (N₂) into ammonia. However, nitrogenases are also extremely sophisticated protein complexes, requiring a plethora of metal-cofactors, large biosynthetic and assembly processes, and harboring important environmental constraints, such as sensitivity to oxygen. The emergence and evolution of the nitrogenase places an interesting evolutionary problem. In this study, we present the evolutionary reconstruction of nitrogenases from their early emergence, around 3 billion years ago. We show that nitrogenases have evolved to adapt to diverse habitable niches on Earth. Using ancestral sequence reconstruction combined with AlphaFold-powered protein structure prediction, we inferred the evolution of nitrogenases from their earliest

tractable ancestors to the modern complex. In total, we predicted over 5,378 structures belonging to 769 nodes of the nitrogenase phylogenetic tree, where we identified key structural insertions that distinguish major nitrogenase clades and potentially influenced their adaptation to changing environmental conditions, including the rise of oxygen on Earth. All structural reconstructions can be accessed via nsdb.bact.wisc.edu. Further, through deep time sequence analyses and functional characterization, we identified a pivotal molecular innovation: the appearance of the G subunit in the last common ancestor of the vanadium- and iron-dependent nitrogenase clade. Our findings highlight the interplay between molecular evolution and ecological transitions, offering new insights into the evolution of globally significant enzymes over long timescales.



Evolution and evolvability of rifampicin resistance across the bacterial tree of life

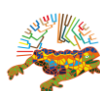
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POSTER SESSION 3 - P03.114, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Many bacteria are faced with lethal stress caused by antibiotics and similar compounds. This includes pathogens and other host-associated bacteria that are exposed to antibiotic treatment. However, given that most current antibiotics are derived from compounds naturally produced by microbes, it also includes many free-living bacteria. Except for the most well studied clinical pathogens and laboratory strains, we know very little about antibiotic resistance evolution of bacterial species in nature. How many and which bacteria are naturally resistant to which antibiotics, and how easy is it for bacteria to evolve resistance? Here, we investigated the intrinsic presence and evolvability of resistance to rifampicin, the first-line drug against tuberculosis, across the bacterial tree of life. We obtained over 18,000 bacterial reference genomes from all major bacterial lineages and extracted their *rpoB* gene encoding the beta subunit of the RNA polymerase, the target of rifampicin. All *rpoB* sequences were then screened against

a mutation panel comprising 57 single amino acid substitutions previously reported to confer resistance to rifampicin in various species, both with respect to their presence and evolvability. Our results demonstrate that rifampicin resistance mutations are intrinsically present in ~8% of species. Those species tend to be phylogenetically clustered but within some groups intrinsic resistance is very heterogeneous. We then determined the evolvability of rifampicin resistance, here defined as the number of resistance mutations that can arise through a single point mutation. This analysis revealed that although the ability to obtain individual resistance mutations varies considerably across species, overall predicted evolvability is high and relatively homogeneous across all our screened species. Our study provides a global picture of the mutational landscape of rifampicin resistance in nature, affording insight into existing observations and guiding future discoveries and possible applications.



Experimental evolution of antimicrobial peptide resistance, and increased sensitivity in the presence of microbial interactions

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POSTER SESSION 3 - **P03.121**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Antibiotic resistance poses a substantial threat to human and animal health as one of the most pressing challenges of the 21st century, with 10 million annual deaths predicted by 2050. To tackle this challenge, we need to better manage use of antimicrobials, as well as develop novel alternatives. Antimicrobial peptides (AMPs) are part of the innate immune defence of prokaryotes and eukaryotes and present promising alternatives. These are diverse small proteins that typically have narrower target ranges than antibiotics. Whilst AMPs have shown some success in effectively targeting pathogens, AMP resistance can evolve following repeated dosing. Resistance patterns, however, appear inconsistent across AMPs and the drivers and constraints of resistance evolution remain to be resolved. Considering that microbes naturally exist in mixed-species communities, microbial interactions are likely to also play a role in AMP susceptibility. We hypothesised that gradual AMP dose increases would select for resistance (in monoculture), with fitness costs of resistance predicted as commonly described for antibiotics. Secondly, in the presence of competitive microbial interactions we predicted increased AMP susceptibility of focal microbial isolates, due to growth inhibitory effects. To test evolution of AMP resistance, we assayed gut bacterial isolates that are considered opportunistic pathogens, including a species of the

Citrobacter genus. Following serial passaging in the presence of sequential increases in AMP concentration (LL-37), MIC (Minimum Inhibitory Concentration) increased greater than 4-fold in independently evolved lineages. Negligible cross-resistance or collateral sensitivity occurred across another AMP (Cecropin A), a peptide antibiotic and an aminoglycoside antibiotic. We will test for fitness effects in the absence of AMP and perform sequencing to determine mechanisms of resistance. Also, we test for negative growth interactions between a set of co-isolated bacteria of the genera *Citrobacter*, *Enterobacter* and *Bacillus* (isolated from a mouse microbiome) via reciprocal supernatant/spent medium growth assays both in the presence and absence of a sub-MIC concentration of an AMP (Cecropin A). We found a combined effect of AMP and supernatant on growth of the AMP-susceptible Gram-negative isolates. This was apparent through an elongation of the lag phase with AMP, that was amplified in the presence of each supernatant treatment. Our results indicate that AMP resistance evolves *in vitro*; however, in the presence of microbial competitors, AMP efficacy is likely to be enhanced. This provides an important basis for further studies of AMP resistance evolution, in the presence of competitive or cooperative interactions in natural mixed-species communities.



Genetic Adaptation to Environmental Change in Evolving Populations

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POSTER SESSION 3 - P03.111, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

How do populations evolve when the environment fluctuates slowly over hundreds of generations? Theoretical models suggest that populations primarily adapt to the environmental optimum through genetic changes rather than relying on phenotypic plasticity. Previous research has shown that populations adapt better to environmental changes when the environment changes gradually, rather than drastically. This is due to accumulation of small-effect mutations. However, it is not yet known how adaptation takes place under gradually prolonged environmental fluctuations. Here we conduct a long-term evolutionary experiment with slowly fluctuating environments using populations of fission yeast founded from two isogenic clones that differ in their mating type. We gradually introduce and intensify environmental stress to a peak, then remove

it at the same rate to return to the original conditions. Additionally, there is a control treatment where the environment remains constant throughout the experiment. By following allele frequency changes by pooled sequencing, we aim to determine whether adaptation occurs and do populations fix compensatory mutations when the environment returns to the original state. Our findings will reveal whether environmental fluctuations promote genetic divergence over stable environments, and does compensatory adaptation, shedding light on how populations genetically respond to slow and reversible environmental change. Lastly, to test whether accumulated mutations actively contribute to adaptation, we will compare the growth rates and competitive fitness of evolved and ancestral populations across different environments.



How turnover shapes the eco-evolutionary dynamics of complex bacterial communities

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POSTER SESSION 3 - **P03.109**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Bacterial communities provide important services. They supply essential resources to animal hosts, drive carbon and nutrient cycling in natural ecosystems, and catalyze chemical reactions across various industries. To maintain their functions, bacterial communities must turn over: fresh nutrients must be supplied and waste products removed. This process often displaces cells and reduces population density — an unavoidable consequence in systems like the gut where microbes are segregated from host tissue, or in bioreactors, which often require dilution to maintain productivity. Despite its centrality, the impact of turnover on bacterial community function remains poorly understood.

Turnover is often a dual force: it governs both nutrient availability and population displacement. While theory predicts that high turnover may constrain niche diversity or favour fast-growing generalists, empirical data on these dynamics is scarce, especially for complex communities over long timescales. Here we describe a fully manipulative experiment that measured how turnover rate affects the functioning and eco-evolutionary dynamics of complex bacterial communities.

We used three distinct heterotrophic bacterial communities sourced from puddles in tree holes of European beech trees. These communities specialize in breaking down leaf litter and represent complex, functionally rich microbiomes. Communities were propagated over eight weeks in batch cultures containing beech leaf extract under two turnover regimes: (1)

full turnover, where culture volumes were replaced with fresh media (affecting both nutrients and population density), and (2) nutrient-only turnover, where culture was centrifuged before partial removal of spent supernatant (leaving the population intact while renewing resources).

To assess community function, we compared ancestral and evolved communities using growth curves and a suite of metabolic enzyme assays. These assays captured changes in overall productivity as well as shifts in trophic structure. In parallel, we isolated and cultured over 200 individual strains from ancestral and evolved conditions to track trait evolution at the strain level. Community composition was monitored through 16S rRNA amplicon sequencing throughout the experiment, and shotgun metagenomics was used at the beginning and end to capture evolutionary shifts in genotypes. A second experiment was performed using *E. coli* K12 under the same conditions to test hypotheses in a reductive genetic background.

Our findings shed light on a largely unaddressed ecological variable—one that is ubiquitous in microbiome engineering and central to the maintenance of long-term community function. By separating the effects of nutrient renewal and population displacement, this experiment addresses the eco-evolutionary consequences of turnover. These insights may improve our understanding of the constraints animal hosts face when controlling their microbiomes and inform strategies for the design of productive, stable bioreactors.



Hypermutation and Fluctuating Stress Levels Can Enable Evolutionary Rescue

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POSTER SESSION 3 - **P03.122**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

When an environmental change leads to a decline in population size, selection on standing or de novo genetic variation can lead to recovery of the population. This process is called evolutionary rescue and is an important mechanism preventing populations from going extinct due to abiotic stressors such as temperature. In an earlier evolution we explored the evolvability of the upper thermal limit in *Pseudoalteromonas haloplanktis* TAC125, a cold-adapted strain of a globally distributed marine bacterium, by slowly increasing the cultivation temperature over the course of 900 generations. While we succeeded in extending the upper thermal limit by one degree to 30°C, the experiment revealed a constrained evolutionary potential of the upper thermal limit. We hypothesised that increasing the mutational supply might lead to this hard limit being surpassed. In this follow up evolution experiment we picked evolved clones from the previous experiment with and without a hypermutator phenotype and subjected them to regularly fluctuating levels of heat stress (2.5°C apart). By gradually increasing the temperatures over 840 additional generations, we were able to reach 32°C, at which point further adaptation stalled. Every time the temperatures were

increased, the evolving populations declined in optical density but slowly recovered with time, indicating repeating waves of evolutionary rescue. Furthermore, populations descending from hypermutator clones showed higher optical densities throughout the course of adaptation to increasing temperatures compared to populations descending from nonmutator clones. While over half of the hypermutator populations acquired additional mutations in genes affecting the mutation rate, only one nonmutator population evolved a hypermutator phenotype. Hypermutator populations on average accumulated 8x more mutations than nonmutator populations. Interestingly, hypermutator populations experienced 14x more mutations rising to fixation than nonmutator populations. Furthermore, we observe parallel genome evolution with genes encoding for membrane-associated proteins in particular showing the exact same mutations in up to a third of all populations. Our experiment exemplifies how a number of evolutionary processes can interact to increase the success of adaptation, a particularly relevant finding in our rapidly changing world.



Linked evolution of gene expression and fitness in fungal populations

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POSTER SESSION 3 - **P03.132**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

All organisms must cope with environmental variability in nature. However, the role of phenotypic plasticity in adaptation to abiotic changes remains poorly understood. To investigate this, we performed an experimental evolution study starting from two clones of the fungal pathogen *Zymoseptoria tritici*. The experiment was conducted in vitro over one year under both stable and fluctuating temperature regimes. Previous analyses focused on gene expression evolution and plasticity. The main findings highlighted (i) pervasive genome-wide changes in gene expression, including in genes located on dispensable chromosomes—typically known for low transcriptional activity—and (ii) evolution toward reduced gene expression plasticity under fluctuating conditions.

We further analyzed these eight experimentally evolved populations by measuring their relative fitness. Most evolved populations outcompeted their ancestor. Surprisingly, all populations with

significant fitness gains exhibited high fitness across both stable and fluctuating temperature environments, suggesting the evolution of generalist strategies. Our current hypothesis is that this generalism may be driven by plasticity at key regulatory genes.

Finally, we observed a correlation between smaller spore size and larger transcriptomic changes. In the two 'fittest' populations, we identified downregulation of a Wee1-related kinase, a highly conserved gene involved in the timing of entry into mitosis. This suggests that either cis- or trans-acting mutations occurred during the evolution, leading to reduced gene expression, and potentially contributing to the observed fitness changes. Altogether, these empirical findings deepen our understanding of the molecular mechanisms underlying the evolution of plasticity in fluctuating environments and highlight both the adaptive potential and complexity of the interplay between gene expression and fitness.



Long-term survival of microbial viruses suggests an evolutionary bet-hedging strategy

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POSTER SESSION 3 - **P03.123**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Survival outside the host is a key factor affecting the fitness of viruses, particularly in stressful abiotic environments. It is well-known that viral survival is often biphasic or 'tailing': the majority of infectious virions decays quickly, while a minority of 'persistent' infectious virions resists decay. We propose that this common survival pattern may represent an evolutionary bet-hedging strategy to bridge periods of host scarcity. To test this hypothesis, we used the 'chloroviruses', phytoplankton viruses that have biphasic decay under stressful conditions (20°C and constant light). We tracked the survival of infectious virions belonging to four chlorovirus strains and produced under four environmental conditions. We confirmed that a subset of infectious virions could resist decay for ≥ 6

weeks, and that the size of this 'persistent fraction' varied across chlorovirus strains. As expected under bet-hedging, the size of the persistent fraction did not respond to environmental cues. In addition, allowing persistent viruses to replicate produced the same mix of nonpersistent and persistent progeny. A final criterion for bet-hedging is that each phenotype is most fit in its respective environment, i.e. that persistent progeny pay a fitness cost when hosts are plentiful. We attempted to test this criterion, but methodological limitations rendered the results inconclusive. Overall our results support the hypothesis that biphasic decay could be a bet-hedging strategy for chloroviruses, and by extension for other viruses.



Multi-layered ecological interactions determine growth of clinical antibiotic-resistant strains within human microbiomes

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POSTER SESSION 3 - P03.128, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Understanding the spread of antibiotic-resistant bacteria in human-associated microbial communities, particularly in the gut microbiome, is critical for combating the global resistance crisis. However, the role of various ecological factors, such as within-species or between-species interactions, in modulating this process is poorly understood. We hypothesized that different strains within the same species would exhibit distinct interactions with the resident microbiota, leading to variable invasion outcomes. To test this, we examined the population growth of multiple clinically relevant and phylogenetically distinct antibiotic-resistant *E. coli* strains carrying ESBL and carbapenemase resistance plasmids. We introduced these strains into human gut microbiome samples from healthy individuals, using replicated anaerobic microcosms treated or untreated with antibiotics. We found that while antibiotic exposure significantly influenced the growth of these incoming strains, some were successful even in the absence of

antibiotics. The variation in ecological success across strain-microbiome sample combinations was positively associated with the intrinsic growth capacities of the strains in local abiotic conditions and their competitive interactions with resident *E. coli*. Furthermore, the incoming resistant strains had strain-specific effects on the taxonomic composition of the resident microbiota; different strains not only varied in their growth performance in human microbiomes, in some cases they pushed taxonomic composition of the resident community in different directions. In contrast with phenotypes measured in gut microcosms, metabolic profiles measured across various single-carbon-source environments were not reliable predictors of population growth in human microbiomes. Overall, this controlled design reveals the multi-layered ecological dynamics and strain-specific mechanisms that govern the spread of antibiotic-resistant bacteria in individual human-associated microbiomes.



Mutation bias predicts rapid emergence of antibiotic resistance

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POSTER SESSION 3 - P03.126, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Rapid and high level antibiotic resistance in bacteria often involves specific mutations in target genes, such as streptomycin resistance via A·T→C·G mutations in rpsL. Therefore, bacteria that sample more A·T→C·G mutations should evolve streptomycin resistance faster. Although rapid sampling of such mutations are relatively rare in wild type bacteria, mutator strains with defective DNA repair genes do have the requisite mutation spectrum, and they indeed evolve resistance faster (Couce et al 2013). However, two questions remain unanswered. First, can this effect of specific mutation bias be generalized across antibiotics? Second, most mutator strains also have a higher mutation rate, which facilitates adaptation. Can we disentangle the role of mutation bias and rate in the context of antibiotic resistance? To address these gaps, we exposed *Escherichia coli* mutator strains with varying mutation rate and spectra to a range of concentrations of antibiotics targeting different cellular

functions, over a single growth cycle. We hypothesized that under strong antibiotic selection, high mutation rate should facilitate adaptation by shortening the time to sample a beneficial resistance mutation. Furthermore, strains whose mutation spectra align with high-benefit resistance mutations in target genes will be more likely to evolve higher resistance. For most antibiotics, strains with the “correct” mutation bias do acquire highest resistance, allowing them to grow at very high antibiotic concentrations. Unexpectedly, these mutations do not arise faster in strains with high mutation rates. Also unexpectedly, the high-benefit resistance mutations were generally less costly. Thus, we find that specific mutation bias is a key predictor of rapid emergence of antibiotic resistance. Given that mutators often arise spontaneously in laboratory, natural and clinical settings, we suggest that their mutation spectra deserve more attention.



Nitrogen availability drives ecological dynamics and parallel evolution in synthetic root-associated bacterial communities.

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POSTER SESSION 3 - **P03.124**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Microbial communities in the rhizosphere—the soil region directly surrounding plant roots—are maintained by complex and dynamic interactions among not only microbial species, but also between microbes and their host plants. These interactions are shaped by environmental factors such as nutrient availability, host plant fitness, and the presence or absence of competitors – and in turn, influence host fitness and nutrient cycling within the system. The availability of nitrogen, a key nutrient for both plants and microbes, strongly influences rhizosphere community assembly, diversity, and function. However, the eco-evolutionary consequences of nitrogen limitation on a root-associated bacterial community remain insufficiently explored. Here, we passaged synthetic bacterial communities inoculated onto canola (*Brassica napus*) seeds in a simplified gnotobiotic system to investigate how nitrogen availability impacts community dynamics and the evolutionary trajectories of individual species.

Materials and Methods: We constructed a synthetic community of 13 bacterial species isolated from field-grown canola roots. Communities were inoculated onto surface-sterilised canola seeds in six independent lineages (n = 12 seeds per lineage) and grown in gnotobiotic microcosms on Murashige and Skoog (MS) agar containing nitrogen compounds for 14 days. After this cycle, seedlings were harvested, root-associated communities recovered, and transferred to fresh seeds for the next cycle. Following six such transfers under nitrogen-replete conditions, seeds were moved to MS agar lacking nitrogen compounds (MS–N) for an additional six nitrogen-scarce cycles. Community population size and composition across all cycles were measured via colony-forming unit (CFU) counts and 16S rRNA amplicon sequencing, respectively. To assess evolutionary responses, individual bacterial clones were isolated from each lineage at the final nitrogen-scarce cycle and subjected to whole-genome sequencing.

Results: Nitrogen removal significantly reduced mean microbial population sizes and increased

variance in population sizes across lineages, indicating reduced microcosm carrying capacity and greater stochasticity under nutrient stress. Notably, alpha diversity increased by the final nitrogen-scarce cycle, suggesting a shift in competitive balance and niche availability. Community dynamics were strongly influenced by the presence or absence of nitrogen: under low nitrogen, *Enterobacter ludwigii* became dominant across all six lineages, while *Klebsiella grimontii*, a known nitrogen-fixer, persisted at reduced but stable frequencies. Other species exhibited fluctuating or declining relative abundances, reflecting altered interspecific interactions under nutrient stress.

Whole-genome sequencing revealed strong signatures of parallel evolution, particularly in *K. grimontii*, where recurrent fixed mutations were detected in genes associated with nutrient stress, across independently evolved lineages.

Our findings demonstrate that nitrogen availability acts as a strong and reproducible selective force, shaping both the ecological dynamics and evolutionary trajectories of root-associated bacterial communities. Nitrogen limitation alters species interactions and community structure, while also driving parallel adaptive evolution in genes associated with nutrient stress.

Conclusion: This study uses a non-model plant species and synthetic bacterial communities to investigate the impacts of nutrient availability on microbial community structure and evolution. Our tractable gnotobiotic system provides a versatile framework for studying eco-evolutionary processes in other non-model plant-microbe system. The most immediate and evident applications are in the agricultural sector, where soil microbiomes may be engineered as bio-inoculants to buffer crucial crop species against the impacts of climate change, and support food security. Our microcosm system may also contribute to conservation efforts by experimentally evolving resident microbial communities associated with threatened native plant species to enhance seedling establishment success during the restoration of native habitats.



Nutrient-rich spatial refuges buffer against extinction and promote evolutionary rescue in evolving microbial populations

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POSTER SESSION 3 - **P03.120**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Microbial populations are often exposed to long-term abiotic disturbances, which can reduce population viability and cause local extinction. Eco-evolutionary theory suggests that spatial refuges can facilitate persistence and evolutionary rescue. However, one drawback of spatial refuges is reduced exposure to nutrients such as carbon and oxygen, suggesting the protective effect of refuges depends on the interplay between environmental conditions and the degree of stress.

Here, we test this general idea using mathematical modelling, and experimental evolution of the model bacterium *Pseudomonas fluorescens* SBW25 under salinity stress. As our model predicted, we find that the ability of spatial refuges to rescue evolving populations from extinction

crucially depends on nutrient availability. Populations evolving under salinity stress where nutrient-rich spatial refuges were available, harboured clones that displayed enhanced salt resistance, indicating that nutrient-rich spatial refuges can facilitate evolutionary rescue. Furthermore, while control-salinity-evolved populations adapted to spatial structure by evolving enhanced motility (likely through parallel mutations in PFLU_4551, a predicted aerotaxis response regulator), this phenotype was constrained under high salinity, because increased motility negates the benefits of a spatial refuge. Our results reveal a general interplay between spatial refuges and nutrient availability that could be leveraged to reduce extinction risk in natural populations.



Role of β -lactamase in cephalosporin resistance evolution in structured environments

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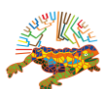
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POSTER SESSION 3 - **P03.116**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Plasmid-borne β -lactamase genes pose problems to the treatment of *Escherichia coli* infections. Selection experiments with bacterial strains are a powerful approach to understand the conditions affecting the role β -lactamases in β -lactam resistance. Yet, most work is done using unstructured liquid cultures, whereas *E. coli* typically grows in structured environments, such as in biofilms.

Aim and methods: We subjected *E. coli* strains expressing blaTEM1-c from single and multi-copy plasmids to selection with 3rd-generation cephalosporin cefotaxime, in broth and on agar, to study the contribution of TEM-1 β -lactamase to resistance evolution as a function of environmental structure.

Results and conclusion: We found that strains evolved higher resistance levels in broth than on agar. TEM activation only contributed to resistance in strains expressing TEM-1 from a multi-copy plasmid, and did so more in broth than on agar. Mutations affecting cefotaxime-target PBP3 play an equally important role in the evolution of cefotaxime resistance across strains in both environments, while mutations that enhance cefotaxime efflux play a more important role in control strains without TEM-1 β -lactamase and more so in broth. Our findings suggest that the adaptation of β -lactamases to novel β -lactams is limited by the supply of mutations, as well as by environmental structure.



Stress-Driven MicroRNA Dynamics in *Phaeodactylum tricornutum*: Unveiling the Molecular Mechanisms of Microalgal Plasticity

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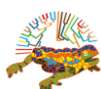
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POSTER SESSION 3 - P03.113, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

MicroRNAs (miRNAs) are small non-coding RNAs that can regulate gene expression post-transcriptionally. They are fine tune modulators that have a pleiotropic effect, interacting with different targets and are deeply embedded in gene regulatory networks. Indeed, their modulation can directly or indirectly regulate pathways without energetically stress the cell. Although, they are widely investigated in several model organisms, they remain poorly studied in marine organism, even in model ones such as *Phaeodactylum tricornutum*. Therefore, we wanted to identify miRNAs in this diatom, observing their differential expression under three stress conditions that could represent changing environments: low salinity, nitrogen limited, and metals contamination. Small-RNAseq, allowed to identify novel miRNAs with the combined use of miRDeep2 and miRDeep-P2. Then, targets

of the found miRNAs have been predicted using miRanda and psRNATarget tools and further analysed via mRNA sequencing in the three conditions. Moreover, the processing and functionality of some miRNAs will be characterized via overexpression of precursors. In the end, a public available database called “miRnutum” will be created, which will contain information about mature and precursor miRNAs, their differential expression in the three conditions, their putative direct targets and gene regulatory network in which they may be involved. We strongly believe that our work will provide novel insights on gene regulation and metabolic pathways in diatoms and will provide new tools for the generation of complementary synthetic biology strategies for biotechnological applications being of support for researchers worldwide to understand diatom’s molecular complexity.



The rate of spontaneous m6A changes in bacteria

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POSTER SESSION 3 - P03.119, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

DNA methylation in bacteria is involved in fundamental processes such as DNA mismatch repair, DNA replication and gene expression. Methylation can be applied to multiple bases, but for bacteria the most common methylation site is N6-methyl-adenine (m6A). The methylation status of individual bases can change spontaneously, and these changes have been shown to have consequences for the cell. The most famous example of these is the phase variation of the pap operon in uropathogenic *Escherichia coli*, where the methylation state of particular adenine bases determines if the cell can grow a pilus to aid in colonization of the urinary tract. Other examples of the impact of m6A methylation for the cell include changes in virulence and sporulation. Despite the importance of m6A methylation to bacteria, the rate at which these methylation changes happen across the genome has not yet been uncovered. Knowing the rate of m6A methylation is important to evaluate its significance to adaptation; if the epimutation rate is very fast, changes in m6A methylation status are unlikely to affect adaptation in the long term, as any effect they might have will be very

short-lived. On the other hand, m6A changes have been shown to be able to affect adaptation in the right conditions. Our study aims to calculate the m6A epimutation rate by combining a mutation accumulation experiment of multiple bacterial lines with third-generation sequencing. Our study species are *Serratia marcescens* and *Escherichia coli*, two strains each. The experiment will include 80 bacterial lines in total, half of which will be grown in a medium with glucose as carbon source, and the other half with glycerol as carbon source. Using these two media will allow us to evaluate the effect of stress on the epimutation rate, as glycerol is less preferred carbon source for the bacteria than glucose. The lines are propagated by taking a single colony from a previous plate and streaking it on a new plate every 2-4 days, depending on the growth rate of the strain. This method will minimize the effect of natural selection on the lines and allow even harmful mutations to accumulate. Then we will sequence these lines at different time points to find methylated sites and calculate their epimutation rate.



The role of evolving niche choice in herbivore adaptation to host plants

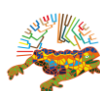
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POSTER SESSION 3 - P03.112, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Individuals living in heterogeneous environments often choose microenvironments that provide benefits to their fitness. Theory predicts that such niche choice can promote rapid adaptation to novel environments and help maintain genetic diversity. An open question of large applied importance is how niche choice and niche choice evolution affect the evolution of insecticide resistance in phytophagous insects. We, therefore, developed an individual-based model based on phytophagous insects to examine the evolution of insecticide resistance and niche choice via oviposition preferences. To find biologically realistic parameter ranges, we performed an empirical literature survey on insecticide resistance in major agricultural

pests and also conducted a density-dependent survival experiment using potato beetles. We find that, in comparison to a scenario where individuals randomly oviposit eggs on toxic or non-toxic plants, the evolution of niche choice generally leads to slower evolution of resistance and facilitates the coexistence of different phenotypes. Our simulations also reveal that recombination rate and dominance effects can influence the evolution of both niche choice and resistance. Thus, this study provides new insights into the effects of niche choice on resistance evolution and highlights the need for more studies on the genetic basis of resistance and choice.



Universal bacterial clade dynamics dominate under predation despite altered phenotypes and mutation targets

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POSTER SESSION 3 - P03.108, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Recent studies have revealed bacterial genome-wide evolution to be complex and dynamic even in a constant environment. The evolution is characterized by the emergence of new clades competing or temporarily coexisting as each clade continues to undergo evolutionary change. Previous studies on predator-prey dynamics tracking simple ecological and phenotypic metrics have shown predation to fundamentally alter prey evolution, often giving rise to defense evolution followed by frequency dependent selection between defended and undefended prey genotypes as well as coevolution. Moreover, these dynamics can differ between prey species depending, for example, on mutation rate and the physiological and fitness effects of the mutations available. Here we sought to

consolidate these fields by examining genome-wide evolution in five bacterial prey species separately subjected to long-term evolution under ciliate predation. For all species, we found mutational signals of prey defense evolution, with phenotypic data demonstrating change in phenotypic composition for defense trait over time. Mutational targets and dynamics also differed between prey species. Intriguingly, however, overall temporal molecular dynamics across the prey species were strikingly similar to those of bacteria evolving alone, with constant emergence, competition and quasi-stable coexistence of clades. This makes molecular evolution more interesting and less predictable than we might expect based on existing coevolutionary theories.



World Enough and Time: Mapping the Martian Adaptive Landscape with a Terran Bacterium

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POSTER SESSION 3 - P03.131, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The present-day surface of Mars is a frigid, UV radiation-soaked desert blanketed by a hypobaric atmosphere. While Terran extremophiles, and even some mesophiles can survive these conditions, none can reproduce, which is a prerequisite for adaptive evolution. But what if cells were allowed to evolve under each of these conditions, then given time to adapt to others? Could evolution rewire the genetic circuitry of a Terran bacterium so that it could not only survive but also replicate? We are using laboratory selection experiments to simulate aspects of the physical conditions in these near-surface environments and gain insight into their habitability. Specifically, we are evolving *Escherichia coli*, a model human commensal, under the following Mars-relevant stressors: freeze-thaw cycles, UV radiation, and desiccation. To follow the adaptive process and to ascertain the fitness of new mutations as they arise, we employ a molecular barcoding approach that enables us to track the frequencies of more than 100,000 barcoded lineages over the course of a short-term selection experiment (~150 generations). This approach will also facilitate the selection of hundreds of putatively adaptive mutants for whole

genome sequencing. Here we describe our experimental design, the sensitivity of our ancestral *E. coli* strain to each of the selective conditions, and report on the preliminary results of these evolution experiments. Our research will address four questions: (1) How, mechanistically, might microbes adapt to each of four physical challenges prevailing on the present-day Martian surface? (2) Which of these challenges presents the greatest obstacle to adaptive evolution, and why? (3) If cells can adapt to Mars-like surface conditions, or to a subset of those conditions, do trade-offs appear when cells are cultured under benign conditions? Finally, (4), do we see correlated responses to selection, i.e., are cells adapted to one stress predictably more resistant – or more susceptible – to others? If we ultimately find that a Terran microbe can evolve a physiology that enables it to reproduce under these conditions, perhaps it is possible that Martian life, if it ever existed, was also able to adaptively evolve when Mars underwent climate change long ago. If so, then near-surface environments such as ephemerally-wetted crater walls may still support microbial life.



A competition-detoxification balance controls the evolution of resistance in simple microbial communities.

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Upon environmental challenges, such as exposure to antibiotics, resistant individuals can emerge and rescue a bacterial population from extinction. Such adaptation to stressful environments has classically been studied in the lab by evolving microbial species in isolation, thus ignoring ecological interactions—a key component of natural ecosystems. The presence of other species can either hinder the emergence of resistance (e.g. by competition) or promote it (e.g. by making the environment more tolerable). To establish a more quantitative understanding of the alternative outcomes, we adapted existing evolutionary rescue theories to account for ecological interactions and generate predictions on the probability of acquiring resistance. We test

the model's predictions through in vitro experimental evolution of simple bacterial ecosystems, where adaptation is relatively fast and ecological interactions can be tuned. Combining mathematical modeling and experimental work we identify a key factor determining the likelihood of the susceptible species to acquire resistance: the balance between competition and facilitation (via environmental detoxification) with the partner species. Our approach brings together ecological and evolutionary processes to gain a fundamental understanding of how resistance (to antibiotics or other toxins) can emerge and spread in species-rich microbial ecosystems.



Adaptation to complex environments reveals pervasive trade-offs and genomic targets with pleiotropic effects

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MEETING ROOM 129+130, August 21, 2025, 10:30 AM - 12:30 PM

Much of our knowledge on the dynamics of adaptation comes from experimental evolution studies that expose populations to a single selective pressure. However, populations in nature rarely adapt to a single stress at a time. Instead, various biotic and abiotic factors come together to produce complex selective environments. Here, we used experimental evolution to describe adaptive dynamics in the presence of simultaneous stressors, and to quantify the evolution of trade-offs between stressors in a complex environment. We adapted populations of the yeast *Saccharomyces cerevisiae* to a full-factorial combination of four stressors over the course of 15 serial transfers. Rapid fitness increases were accompanied by the accumulation of mutations in genes and pathways related to specific stressors. Trade-offs evolved rapidly, with the order and mode of trade-off evolution varying between environments due

to the inherent physiological and genetic basis of resistance to each stressor. As environmental complexity increased, mutations had increasingly broader effects across multiple biological processes. These pleiotropic mutations targeted regulatory mechanisms such as transcription factors and chromatin modifiers, which control gene expression networks beyond those directly involved in individual stress responses. Although shared mutations at the individual SNP level were rare, recurrent mutations affecting the same genes and putative biological processes were abundant. Our results suggest that adaptation dynamics in simple environments cannot be extrapolated to predict evolutionary patterns in complex environments with multiple simultaneous stressors, as fundamentally different adaptive processes emerge when organisms face combined selective pressures.



Adaptation to multiple simultaneous stresses leads to a more costly and easier to reverse resistance when compared with adaptation to stresses added sequentially

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Bacteria are frequently subjected to multiple antibiotics in many ecosystems they inhabit, including in a healthcare setting. Exposure may vary based on differences in treatment, with bacteria commonly treated in a sequential manner, i.e. first trying one antibiotic then adding another later in the treatment. More recently the idea of combining multiple antibiotics as part of a treatment has gained traction. It is expected that the more complex “combination” treatment will lead to a more general resistance mechanisms with a higher fitness cost, while a “sequential” treatment is expected to be less costly and more specific to the antibiotics the bacteria are exposed to. However, these hypotheses for treatment outcomes remain understudied, with important implications from a human health perspective, as well as being an interesting evolutionary question.

We have experimentally evolved multiple replicate populations of the bacterium *Pseudomonas aeruginosa* to three distinct antibiotic stressors simultaneously, resulting in multidrug resistant bacteria. We used two treatment regimes: combination (where all antibiotics are added simultaneously) and sequential (where the three antibiotics were added in a sequential manner). Surprisingly, after experimental evolution the two treatments resulted in similar levels of resistance both to the experimental antibiotics as well as in cross-resistance. However, as predicted the combination treatment resulted in lower fitness (median of 48.5% reduction in growth rate) when growing in an antibiotic-free environment than the sequential treatment (median of 12.3% reduction in growth rate). Genomic analysis of resistant clones revealed that adaptation in the sequential treatment was driven by specific mutation related to resistance to the experimental antibiotics, with genes involved in

efflux pumps frequently targeted. Conversely, combination treatment often resulted in mutations of *parS*, a high-level regulator controlling expression of multiple genes involved in antibiotic resistance. These phenotypic and genomic patterns were repeated across two distinct strains.

We were also interested in how the different evolutionary histories of our experimentally evolved samples could lead to different evolutionary trajectories when the antibiotic stress was removed. More specifically, will the high cost more general resistance in the combination treatment mean that increases in fitness will be reliant on loss of resistance? So, next we experimentally evolved our previously adapted and sequenced clones for 12 days in the absence of antibiotics. Preliminary results (May 2025) suggest a rapid increase in fitness, with many samples reaching near wildtype fitness is combined with a loss of resistance in combination treatment samples. Sequential treatment samples retained showed a more modest increase in fitness, while retaining more of their resistance. We are also sequencing clones from these treatments to uncover the mechanisms behind these rapid changes, with results expected for August 2025.

Overall, our work shows the differences in the outcomes of adaptation to simple and complex stresses. Specifically, that complex treatments can lead to higher fitness costs in the absence of stress even while leading to similar resistance levels. These differences in evolutionary history also directly impact the evolutionary trajectories when stress is removed. This work can help inform our understanding of how bacteria evolve in response to different treatments in a clinical setting, as well as more generally understand how bacteria evolve to multiple stressors.



Assessing the impact of resource availability on plasmid costs and persistence in microbial communities

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MEETING ROOM 129+130, August 21, 2025, 2:00 PM - 3:30 PM

Plasmids are central players in bacterial evolution, allowing rapid adaptation by transferring ecologically and clinically relevant traits. However, plasmids do not necessarily need to be beneficial for their bacterial host, and their maintenance is often unstable in the absence of selection. While environmental context is known to modulate plasmid-associated fitness effects, the effect of nutrient availability, a key ecological factor, on plasmid fitness effects remains largely unexplored. In microbial communities, resource limitation and competition are ubiquitous and can strongly shape evolutionary trajectories. Previous studies have shown that nutrient-rich or minimal media can shift plasmid fitness effects from negative to neutral or even beneficial, or vice versa. However, it remains unclear whether community-driven resource competition can modify plasmid-associated fitness effects to the extent that it explains variability in plasmid dynamics in microbial communities.

Here, we integrate experiments and mathematical modelling to test whether nutrient-dependent plasmid fitness effects are a main driver of plasmid dynamics in complex microbial settings. Using the

OMM12 synthetic gut microbial community, a stable and reproducible model with 12 species that preserves essential ecological complexity, we track plasmid dynamics in three focal host strains (*Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter freundii*) embedded within the community under defined nutrient regimes. We estimate plasmid-associated fitness effects in monoculture and measure growth across isolates and the full community to parametrize an ecological model. The mathematical model allows us to assess whether the observed plasmid behaviour in the community setting can be mechanistically explained by resource-dependent plasmid fitness effects driven by competition for shared resources.

Our study is one of the first to explore and assess the impact of resource-dependent plasmid fitness effects on the plasmid ecology within complex microbial communities. By using an experimentally tractable microbial community and a theoretical framework, this work helps to understand the ecological rules that govern mobile genetic element dynamics in complex microbial communities.



Do heat extremes and drought predictably alter the function and composition of soil microbial communities?

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MEETING ROOM 129+130, August 21, 2025, 10:30 AM - 12:30 PM

Background: Climate change introduces a range of environmental stressors, including heat extremes and droughts, which affect species differently. For soil microbes, which exhibit remarkable resilience, the effects of these stressors may vary in terms of both recovery mechanisms and predictability. While some microbial community changes after such extremes may be stochastic, others may follow more predictable trajectories. Crucially, the temporal scale over which these responses unfold is key to understanding the predictability of community dynamics. Especially in the immediate aftermath of the climactic event. There is also an incomplete understanding of how heat and drought affect communities in the long term. Many studies suggest that climate extremes may increase the frequency of certain genes within the community such as those related to dormancy, sporulation certain kinds of metabolism and even antibiotic resistance genes. As microbial communities provide critical functions in the environment, we wonder how predictable are microbial community changes at different time scales following climate extremes, and how do these changes relate to microbial functions, including metabolism and antibiotic resistance?

Materials and Methods: To address this question, we established the Hasli Outdoor Mesocosm Experiment (HOME), a long-term outdoor mesocosm study designed to simulate climate change impacts on communities including plants, invertebrates and microbes. Forty mesocosms, each seeded with a diverse array of nine plant species (including one invasive species) from Switzerland, were subjected to four temperature treatments: (1) Ambient, (2) Ambient +2°C, (3) Ambient + Heatwaves, and (4) Ambient +2°C with Heatwaves. Over two years, we collected soil cores from the top 10 cm across three seasonal time points and performed Illumina Short-Read Shotgun Sequencing on each sample. We used the SEED subsystems database and the SAMSA2 pipeline to generate

gene annotations and applied linear mixed models to assess treatment effects on gene abundances and community composition of microbes. Additionally, we performed metagenome assemblies with MetaSpades and the MetaPhlan 4 pipeline and compared the frequencies of gene types and microbial OTUs one month and three months after drought treatments.

Results: Our analyses revealed an increase in genes related to sporulation, dormancy, and antibiotic resistance under heat stress, with a clear and consistent response in the short term (one-month post-treatment). Other genes related to metabolism decreased over time in the heat stress treatments. In contrast, the presence of drought did not produce similar changes in these gene types, typically remaining relatively unaffected. Additionally, we observed that microbial community changes—both in terms of gene abundances and community structure—were more predictable in the first month following heat stress than at three months, where the responses became less consistent. Importantly, the seasonal context and other biotic and abiotic factors also played a significant role in shaping these community dynamics.

Conclusions: Our findings suggest that microbial responses to heat extremes are both predictable and consistent over short time scales, particularly in relation to stress-related genes such as those involved in sporulation and antibiotic resistance. However, as time progresses, the predictability of microbial community changes diminishes, highlighting the complexity of long-term microbial responses to climate change. Understanding the temporal dynamics of these responses, especially in the face of multiple interacting environmental stressors, is crucial for predicting the future evolution of microbial communities and their functions under climate change.



Experimental evolution of thermal performance in phage communities

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MEETING ROOM 129+130, August 21, 2025, 10:30 AM - 12:30 PM

Species evolution depends on a combination of biotic and abiotic selection pressures which often interact with each other. Global climate change is expected to alter these selection pressures through rising environmental temperatures (abiotic) and changes to community compositions and host-parasite dynamics (biotic). As a result, climate change is hypothesised to alter evolutionary trajectories by modifying the strength or direction of interactions between biotic and abiotic pressures. In this study, we use experimental evolution with a bacteria-bacteriophage (phage) system to investigate the evolutionary interplay between an abiotic selection pressure (temperature) and a biotic selection pressure (competition). Phages were evolved under two thermal

regimes (37°C or 42°C) in the presence or absence of a phage competitor. Phage evolution was assessed through phenotypic tests and population sequencing of end-point evolved lines. We show that phages rapidly adapt to their thermal environments with higher fitness found in evolved vs naïve temperatures. Phenotypic changes were linked to genetic divergence between high and low temperature-evolved lines and mutations involved in phage DNA transport. We further show that competition constrains evolution to temperature by reducing growth, and in turn evolution rates. Our findings suggest that antagonistic biotic interactions, such as competition, may limit adaptation to abiotic stressors, potentially increasing species vulnerability to climate change.



Genetic local adaptation of gut microbial species in metabolically and structurally distinct intestinal compartments

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MEETING ROOM 129+130, August 21, 2025, 2:00 PM - 3:30 PM

A key question in population genetics is understanding which genes enable populations to thrive in their local environments—a phenomenon known as (genetic) local adaptation. While local adaptation has been extensively studied in plants and animals, much less is known about how microbes adapt genetically to their local environments in nature. Existing microbial local adaptation studies often focus on adaptation between host and symbiont or between populations isolated across large geographic distances. In contrast, less is known about microbial local adaptation at small spatial scales within well-connected populations.

Theory predicts that the large local population sizes of microbes may strengthen their response to selection, which should promote local adaptation. On the other hand, high dispersal rates and clonal reproduction may erase differentiation and thus inhibit local adaptation. Additionally, commonly used population-genetic methods may not be fully applicable due to factors like strong selection and non-independent loci.

To explore microbial local adaptation at fine spatial scales, we studied gut microbiome communities in seven wild roe deer (*Capreolus capreolus*), sampled at 10 locations along the gastrointestinal tract. Treating each gut region as a discrete but connected habitat—akin to a stepping-stone model—we generated ultra-deep

metagenomic data, yielding ~60 million paired-end reads per sample across 70 samples. De novo metagenomic assembly revealed highly diverse microbial communities, including many previously undescribed lineages.

Focusing on 20 highly covered species from the hindgut (cecum and rectum), we found that 50% showed significant signatures of genetic local adaptation. These signals were especially striking given that community composition between these gut regions was nearly identical—suggesting that compositional similarity does not necessarily reflect evolutionary or functional equivalence.

We propose two main drivers of this local adaptation: divergent regimes in nutrients or adaptability. In several species, we identified candidate local adaptation loci within mobile genetic elements such as transposases or integrases, often nearby significant signatures of gene transfer, suggesting that the rate of gene transfer itself may be the trait under local adaptation. Furthermore, our findings point to horizontal gene transfer as a key mechanism enabling microbial adaptation in the face of high levels of incoming migration.

Overall, our study highlights the potential for genetic local adaptation even in highly mixed microbial communities and emphasizes the importance of fine-scale environmental variation in shaping microbial evolution.



Heteroresistance as an adaptive strategy of *Pseudomonas aeruginosa* in fluctuating antibiotic environments

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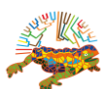
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Background: Heteroresistance (HR) is described as the presence of a resistant subpopulation within an predominantly susceptible isogenic bacterial culture. It enables a fraction of cells to survive transient high antibiotic concentrations and can serve as a stepping-stone toward stable resistance. HR has been observed across diverse pathogens and may compromise both diagnostics and antibiotic therapy. To date, it is unclear which conditions favor the evolution of HR. We here tested the idea that HR provides a selective advantage under fluctuating antibiotic conditions.

Materials and methods: A long-term evolution experiment was performed with *P. aeruginosa* PA14, including several fluctuating treatment designs involving frequent switches between three antibiotics with distinct cellular targets (ciprofloxacin (DNA gyrase), gentamicin (ribosome), and carbenicillin (cell wall)), and then corresponding monotherapies without any switches. Populations were propagated across 96 treatment steps. Individual clones were isolated and characterized for their resistance and HR (using the population analysis profile (PAP) test) after 12, 48, and 96 steps. Whole-genome sequencing of representative HR isolates was conducted to identify mutations associated with adaptation. We used competition experiments to assess under which antibiotic treatment conditions HR provided a selective benefit.

Results: We identified an increased rate of HR evolution under fluctuating antibiotic treatments, especially those with fast, random drug switches. In subsequent competition experiments, different HR isolates outcompeted the ancestral strain under complex switching conditions and specifically when challenged with gentamicin. This indicates that HR confers a fitness benefit in both a specific drug environment (constant gentamicin) and in dynamically changing drug environments. Genomic analysis of evolved HR clones revealed multiple mutations in genes encoding transcriptional regulators of efflux pumps. Notably, mutations in *mexZ* gene and mutations in *nalD* gene were prevalent among HR variants suggesting that HR evolves through antibiotic efflux. Intriguingly, the resistant subpopulations characteristic of HR were often unstable in the absence of antibiotics and thus, the proportion of highly resistant cells in HR isolates diminished over time.

Conclusions: Our findings demonstrate that dynamic antibiotic environments accelerate the evolution of HR in *P. aeruginosa*. HR may thus provide a bet-hedging strategy, allowing bacterial populations to rapidly adjust to fluctuating selective pressures. These results have important implications for antibiotic therapy design and advance our understanding of microbial adaptation to changing environmental conditions.



Phenotypic heterogeneity in capsule production across opportunistic pathogens

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MEETING ROOM 129+130, August 21, 2025, 10:30 AM - 12:30 PM

Phenotypic heterogeneity allows bacteria to adapt fast to changing environments. Extracellular surface structures are the primary interface between the cell and environment. They are main drivers of bacterial adaptation, horizontal gene transfer and host-pathogen interactions. One of such structures, the polysaccharidic capsule is present in ~50% of species across the bacterial phylogeny, including all ESKAPE microorganisms, the six most significant multi-drug resistant (MDR) nosocomial pathogens. It is also a major virulence factor. However, capsule production is a costly trait under certain environmental conditions. To offset the cost of its production, some capsulated bacteria resort to genetic phase variation mechanisms, which relies on point mutations or slipped-strand mispairing. Yet these mechanisms are not optimum in rapidly changing environments as they require a lag time for adaptation.

Here, we reveal the existence of a broad novel mechanism of non-genetic phenotypic heterogeneity in the production of the bacterial capsule in different *Klebsiella* and *Acinetobacter* species. We developed a solid, reproducible, agnostic and unbiased methodology to determine heterogeneity in capsule production using density-based separation by gradient centrifugation. Analyses of more than 180 strains in four different environments varying in resource availability and spatial structure, reveal that that 71% of *K. pneumoniae* strains can be heterogeneous. Such heterogeneity is not an ON/OFF state but rather responds to a discrete continuum of capsulation resulting in mixed populations of hyper-, hypo- and intermediate capsulated cells.

Our genomic analyses show that the distribution of heterogeneity is associated with certain lineages of *K. pneumoniae*,

prone to acquire antimicrobial resistance, but not with hypervirulent *K. pneumoniae* strains encoding the hypervirulent regulator, *rmpA*. Distribution of heterogeneity across the phylogenetic tree revealed that it does not fit a model of Brownian evolution, suggestive of adaptive evolution of heterogeneity. Indeed, we show that when competing against non-capsulated mutants, heterogeneous strains are fitter than non-heterogeneous strains. This heterogeneity responds to environmental cues, and changes in capsule production at the population-level result from more subtle changes in the proportion of hyper- and hypo- capsulated cells within the population. Further, heterogeneity impacts interactions with mobile genetic elements, for instance, it allows a certain subpopulation to escape from phage predation by limiting phage adsorption.

Despite the epigenetic regulation underlying capsule phenotypic heterogeneity, we show that it depends on specific capsule operon sequences, as exchanges of the capsule operon across strains, also alters the population heterogeneity.

Taken together, our results help understand the success of *K. pneumoniae* in the transition from healthy colonization, where limited capsulation is required, to systemic infection, conditions in which high levels of capsulation are advantageous. Phenotypic heterogeneity in capsule production could correspond to a bet-hedging mechanism which impacts adaptation directly by allowing a subset of the population displays different susceptibility to different abiotic and biotic stresses. Such heterogeneity can also indirectly impact adaptation via horizontal gene transfer by driving interactions with mobile genetic elements.



Unravelling the evolutionary and ecological impacts of multiple agricultural stressors on soil microbial communities

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MEETING ROOM 129+130, August 21, 2025, 10:30 AM - 12:30 PM

Soil microbial communities are key drivers of agricultural processes such as nitrogen provisioning, protecting crops from pathogens and heavy metal bioremediation. However, such communities are increasingly exposed to multiple harsh environmental conditions imposed by anthropogenic activity such as antibiotic run-off from animal farms, the application of pesticides, and increasing salinity and temperatures due to climate change. Here, we test whether multiple stressors pose distinct challenges for microbial adaptation relative to stressors imposed individually, at both the population and community levels.

First, we investigate how the plant growth-promoting rhizobacterium *Pseudomonas fluorescens* responds to various multiple stressor scenarios (fungicides and increasing temperatures) over evolutionary timescales. We find that *P. fluorescens* can rapidly adapt to medium-high fungicide concentrations, under both control and temperature stress. However, when both stressors are imposed simultaneously, evolved fungicide resistance mechanisms are not sufficient to prevent population extinction. Genome resequencing suggests that *P. fluorescens* evolves fungicide resistance via the upregulation of efflux pumps and downregulation of porin

expression. Resistance to fungicides correlated with cross-resistance to multiple antibiotics, including chloramphenicol, sulphatriad, nalidixic acid, and tetracycline - highlighting fungicide use as a driver of antibiotic resistance in agricultural ecosystems. In natural soil communities, we found that multiple stressors synergistically drive a decline in soil bacterial community diversity, productivity (soil respiration), and metabolic capacity. Under the multi-stressor treatment, communities typically had reduced α -diversity, decreased productivity, and lost the ability to metabolise key carbohydrates and carboxylic acid-based substrates. Taxa isolated from evolved communities displayed increased resistance to fungicide - as predicted from our experiment using *P. fluorescens*. However, in communities, increased fungicide resistance was not correlated with resistance to antimicrobials - suggesting an alternative mechanism of fungicide adaptation in these non-model organism species.

Our research contributes to the growing evidence that multiple stressor conditions present distinct challenges for microbial adaptation, and suggests that the overuse of biocides, including fungicides, can drive the emergence of antimicrobial resistance.





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S35 - Mechanisms, barriers, and impacts of horizontal gene transfer across the Tree of Life



Addressing the role of horizontal gene transfer in plant-insect interactions

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POSTER SESSION 3 - P03.135, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Horizontal gene transfer (HGT) allows organisms to acquire genes through ways other than reproduction. While common in prokaryotes, its role in eukaryotes—particularly insects—has been less studied, partly due to concerns over contamination or phylogenetic artifacts. However, recent genomic evidence shows that some horizontally acquired genes in insects contribute to herbivory by enabling the digestion of complex plant compounds and the detoxification of plant defenses. These genes may originate from prokaryotic or eukaryotic donors. This PhD project investigates the extent, evolutionary timing, and functional roles of HGT in insects.

Materials and Methods: To support this research, we developed TaxTrack, a tool designed to rapidly detect HGT events across genomes in a systematic way. TaxTrack identifies candidate genes of plant, fungal, bacterial, or viral origin through protein sequence analysis and phylogenetic reconstruction. The pipeline involves four main steps: (1) inference of the last common ancestor (LCA) for each gene; (2) filtering out candidates likely resulting from contamination or transposable elements; (3) homologous protein searches; and (4) phylogenetic tree construction to infer gene origins and transfer events.

Results: To assess the performance of TaxTrack, we benchmarked it on the genome of the whitefly *Bemisia tabaci* (Hemiptera, Aleyrodidae), in which 52 plant-derived genes have been previously identified.

TaxTrack successfully recovered all 52 plant-like genes, along with 3 additional candidates not detected by its earlier version (2022)¹, and 28 more than reported by Li et al. (2022)². Compared to Danchin et al. (2024)³, we identified 4 additional putative plant-like genes, potentially representing novel HGT events. For bacterial-origin genes previously reported by Chen et al. (2016)⁴, TaxTrack retrieved 37 out of 64 candidates. Among the remaining 27: 9 showed similar phylogenetic placement at the root of the bacterial clade; 7 were also placed at the root, though previously classified within the bacterial branch; and 11 were not recovered by our pipeline.

Conclusions: We will extend the use of TaxTrack to a large-scale analysis of 416 insect proteomes representing 21 of the 29 recognized insect orders. This will allow us to estimate the timing of HGT events by analyzing phylogenetic distributions in relation to insect divergence times, and to annotate the functions of transferred genes to evaluate their ecological and adaptive relevance. This study will advance our understanding of the evolutionary role of HGT in insect diversification and adaptation.

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4. Chen, W. et al. *BMC Biol* 14, 110 (2016).



Ctrl+Alt+Conserve: Transformation Reduces Genomic Disparity in Digital Organisms

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POSTER SESSION 3 - **P03.144**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Genetic exchange between and within species is a fundamental evolutionary force. Eukaryotic sex and transformation (acquiring and integrating environmental extracellular DNA) share many benefits and costs, but are highly distinct mechanisms. Several hypotheses attempt to explain the role of transformation in bacteria and archaea - including increased adaptation and innovation akin to sex. Conversely, transformation has been predicted to act as a conservative force due to the discovery of multiple restriction mechanisms that can limit exchange primarily between clonemates. Conflicting results in living organisms make exploring questions surrounding the drivers and genomic effects of differing gene exchange ideal candidates for simulation approaches. Here, we add transformation to the eco-evolutionary

model REvoSim to compare how mutational pressures impact the maintenance of contrasting methods of gene exchange. In addition, we assess their effects on genomic evolution over macroevolutionary timescales. Preliminary results indicate that higher levels of transformation and sex can be beneficial in reducing the mutational load in populations but are costly when the mutation rate is low within digital ecosystems. Genomic disparity is limited by transformation in contrast to sex, supporting the idea that some forms of lateral gene transfer promote stability in vivo. Our results highlight multiple roles acting within gene exchange mechanisms in bacteria and archaea, and align with theoretical predictions that suggest that the eukaryotic sex is adaptive to temporally changing ecological environments.



Detecting non-vertical inheritance across eukaryotes

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POSTER SESSION 3 - P03.145, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

An increasing number of recent studies report a substantial degree of discordance between species phylogenies and the evolutionary histories of their own genes, even in eukaryotic datasets. This incongruence can arise from multiple factors, including analytical errors, differential duplications and losses and, critically, non-vertical evolutionary processes such as hybridization or horizontal gene transfer (HGT). While the extent of HGT in eukaryotes remains a topic of active debate, the expanding availability of genomic data and methodological advances now offer an opportunity to systematically assess its impact.

In this project, we developed a reproducible pipeline coupled with representative databases to investigate HGT across Eukaryotes using two complementary approaches. First, we investigated intra-

clade HGT events through a gene tree-species tree reconciliation framework. Second, we characterized inter-domain HGT events by screening different proteomes in a tree of life scale database. As an initial case study, we applied this framework to Amoebozoa. We then intend to apply this pipeline across all other suitable eukaryotic groups. This could be especially interesting in clades where HGT is known to have an important role in genome evolution.

Ultimately, given the taxonomic incompleteness, potential contamination and different analytical errors, our project endeavors to establish an updatable and reproducible framework which may serve as a foundation for future research efforts, facilitating the integration of new genomic data and the refinement of analytical techniques as they become available.



Giant Virus Heredity is Surprisingly Vertical: Reconciliation of Nucleocytoviricota Phylogeny

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POSTER SESSION 3 - **P03.141**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Nucleocytoviricota is a phylum of double-stranded DNA viruses, also known as Nucleocytoplasmic large DNA viruses (NCLDV), which includes members that infect diverse eukaryotic taxa, from amoebozoans to mammals. One characteristic of their biology is the large genomes and particle size, reaching more than 2.5 Mbp and 2 μ m, respectively. These values are significantly greater than those found in other viral lineages which are typically smaller than 0.2 μ m and harbor more diminutive genomes. They also resort to the creation of a viral factory, an organelle-like transient structure for the reproduction of the virus. Even though they deviate from the expectations given their size, NCLDVs still hold viral characteristics, such as the lack of apparatus for protein translation, instead relying entirely on host cellular resources. The abundance of genetic information in the form of genome availability and multiplicity of proteins have allowed for a robust phylogenetic analysis, showing them to be a monophyletic group.

With a reference set of 1382 nonredundant genomes, and 8793 gene families, we used AleRax, a probabilistic tool for reconciling gene trees with the species tree to evaluate

the propensity for horizontal gene transfer of NCLDV gene families. In this work we show that, in spite of viruses being mobile genetic elements, NCLDV exhibit more vertical gene inheritance than Bacteria. Naturally, that is a reflection of lower propensity for transfers of the gene families of the virus phylum. The same analysis was conducted for phages with large genomes (>200kb), since they share similar biological aspects with NCLDV, with the caveats that large phages do not form a monophyletic group with similar genomic characteristics, and a lower number of genomes and gene families were used (231 genomes and 1309 families). Large phages exhibited even higher verticality.

Given such strong vertical inheritance, one may expect the common ancestor of the NCLDV to have a large conserved genome, yet; on average; less genes are present in the root of the NCLDV species tree than in the root of the large phage trees. A next step in understanding the genomic make of the extant NCLDV, is to look at the hosts phylogeny, the whole eukaryotic domain. This, in turn, should enlighten not only our knowledge on Nucleocytoviricota origin, but also their possible impact on eukaryogenesis.



Host-parasitoid relationship as a driver of horizontal transfer

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POSTER SESSION 3 - P03.137, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Traditionally, evolution has been represented by trees branching out, portraying the vertical transmission of genetic material from parents to offspring; but the transmission of genetic material can also take place across inter-species barriers, a phenomenon known as horizontal transfer. Although predominantly occurring among unicellular procaryotic organisms, horizontal transfer is increasingly documented in multicellular eukaryotes, thanks to the generalization of comparative genomics to non-model species. Horizontal transfer now appears to be a common phenomenon, phylogenetically widespread and responsible for a significant part of the evolution of these organisms.

However, the molecular and ecological mechanisms underlying these horizontal transfers, and their adaptive implications, are still largely unknown. It is now necessary to quantify these events on large taxonomic scales, and to identify their explanatory factors.

The numerous cases of horizontal transfer recorded between host organisms and their parasites suggest that ecological connectivity could affect the frequency of this phenomenon - but to what extent? To address this question, we have chosen to

focus on a particular but widespread ecological relationship amongst insects: the host-parasitoid relationship.

Our project benefits from a combination of extensive ecological and genomic data, derived from decades of fieldwork: we analyzed the complete genomes of 222 insect species, comprised of Hymenopteran and Dipteran parasitoids and their Lepidopteran hosts, which constitute an exhaustive network of ecological interactions within a well-known phylogenetic framework.

Through sequences clustering and phylogenetic approaches, we were able to extensively document horizontal transfer events between independent pairs of host species and their parasitoids, and to assess the contribution of their relationship on horizontal transfer events.

Our preliminary results do suggest a positive effect of the ecological relationship on the frequency of horizontal transfer, especially between hymenopteran parasitoids and their lepidopteran hosts. This study reveals the host-parasitoid relationship as a key driver of horizontal transfer, emphasizing the central role of ecological interactions in shaping eukaryotic evolution.



Long-term selection on mitocoding genes buried in mammalian and avian nuclear genomes

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POSTER SESSION 3 - P03.138, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Sporadically genetic material that originates from an organelle genome integrates into the nuclear genome. However, it is unclear what processes maintain such an integration over longer evolutionary time. Recently, it was shown that nuclear DNA of mitochondrial origin (NUMTs) may harbour genes with intact mitochondrial reading frames despite the fact that they are highly divergent from the host's mitochondrial genome. Two major hypotheses have been put forward to explain the existence of such mito-coding nuclear genes: (A) recent introgression from another species and (B) long-term selection. To address whether these intriguing possibilities do play a role, we scanned the genomes of more than 1,000 avian and mammalian species for NUMTs. We indeed

identified that the subclass of divergent NUMTs harbouring mitogenes with intact reading frames is widespread across mammals and birds. We can show that for these NUMTs signatures of cross-species introgression are widespread in birds, but not in mammals with the exception of ungulates. We can also show that a substantial fraction of deeply divergent NUMTs are maintained by selection. For a small number of NUMT genes, we identify an evolutionary signature consistent with adaptive evolution, including one human NUMT that is shared among seven ape species. This highlights the intriguing possibility that NUMT insertions may occasionally be functional.



Most laterally acquired genes in grasses degrade due to the loss of utility

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POSTER SESSION 3 - **P03.134**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Lateral gene transfer (LGT) is widespread in eukaryotes, including in animals and plants where it can fuel evolutionary adaptation and innovation. However, the proportion of LGT events that are adaptive, and the factors influencing their persistence, remain unclear. Here we use Alloteropsis as a model system to study the evolutionary fate of grass-to-grass transfers. By analysing transcriptome and genome data, we show that a majority of laterally acquired genes appear to be degenerating, being expressed at lower levels and/or more truncated than their vertically inherited ortholog. DNA methylation correlates with this pattern of degradation, with laterally acquired genes overall exhibiting higher methylation levels than vertically inherited orthologs, and degenerating laterally acquired genes having higher methylation than those that appear

functional. For the putatively functional laterally acquired genes, the expression level is generally more similar to the ortholog in the donor species than to the vertically inherited ortholog in the recipient. Overall, the results indicate that most laterally acquired genes detected in a genome are likely to be neutral or deleterious, and therefore the actual background rate of transfer is likely to be many times higher as most laterally acquired genes are rapidly silenced and lost. Those that are retained may encode their own regulation (e.g. presence of adjacent cis-regulatory elements), with expression patterns similar to the gene in the donor species. For a laterally acquired gene to be maintained in the recipient's genome it needs to be readily usable and to offer an evolutionary advantage.



Plasmid distribution reveals genomic determinants for their host range

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POSTER SESSION 3 - **P03.142**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Plasmids play a vital role in bacterial evolution and adaptation, notably facilitating the spread of antimicrobial resistance (AMR) through transfer across hosts. While plasmid host range is a key determinant of their ecological impact, comprehensive investigations of this trait across diverse plasmid populations remain limited. This study aims to characterize plasmid host range, identify features distinguishing broad host range plasmids from narrow host range plasmids. We analyzed over 200 thousand complete plasmid sequences, coupling the plasmid taxonomy unit classification scheme to generate 13 thousand plasmid clusters. Examining the distribution of the clusters across bacterial hosts allows us to

rank plasmids based on their host range. Comparative analysis reveals that broad host range plasmids differ from narrow host range plasmids in plasmid replication mechanisms, mobility, and antibiotic resistance genes. Furthermore, broad host range plasmids exhibit greater variance and a larger mismatch in nucleotide composition compared to the host genomes, suggesting an enhanced tolerance for diverse genetic backgrounds and greater adaptability during inter-host transfer. These findings offer insights into the multifaceted factors influencing plasmid host range and provide a foundation for developing models to predict host range based on plasmid genomic features.



Plasmids in isolation vs plasmids in congregation – how do evolutionary dynamics differ?

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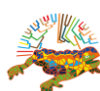
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POSTER SESSION 3 - P03.143, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Plasmids play a significant role in microbial evolution and adaptation by carrying and transferring genes between bacterial hosts. While under selective pressure, plasmid-encoded traits—such as antibiotic resistance—can provide an advantage to their hosts, in the absence of positive selection, plasmids often impose a fitness cost, making their long-term persistence an interesting puzzle in evolutionary biology. One important aspect of plasmid biology that has remained largely unexplored is the interaction between co-infecting plasmids within the same host cell. These interactions can impact the fate of individual plasmids and their hosts. For example, experimental data show that plasmid costs can be alleviated when they are present in a community. Additionally, bioinformatics

analyses also show evidence of multiple plasmid coexistence in one host.

Here, we combine experimental data on bacterial growth and plasmid transmission with mathematical modeling in order to understand factors that contribute to plasmid success, both in isolation and in a community of other plasmids. To do so, we fit mathematical models to bacterial growth curves to quantify key biological properties that influence plasmid persistence and transmission. These include the costs of plasmid carriage, the conjugation rate and the segregational loss rate. This approach adds a new layer of understanding of plasmid dynamics and will ultimately help us make predictions about evolution of these mobile elements in the long run.



Unwelcome guests: characterizing the genomic niche of insertion sequence elements in prokaryotic genomes

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POSTER SESSION 3 - **P03.139**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Insertion sequences (ISs) are a class of minimal, autonomous transposable elements (TEs) found in the majority of prokaryotic genomes. ISs can move within a genome and be transferred between hosts via horizontal gene transfer. Upon transposition, they generate structural variations in genomes, which are often detrimental to the host, though they can occasionally result in beneficial mutations. For instance, they can modulate functional elements such as gene promoters and transcription terminators but also facilitate the horizontal transfer of antibiotic resistance genes. The antagonistic properties of ISs, combined with their highly dynamic nature, make their overall impact on bacterial evolution and genome biology difficult to grasp, with key questions remaining unresolved: 1) Which genomic features of chromosomes constrain the insertion and evolutionary persistence of ISs? 2) To what extent do insertion and persistence depend on the characteristics of the IS element itself?

To address these questions, a promising approach comes from the field of transposon ecology, which views TEs as independent entities inhabiting host genomes and potentially interacting through ecological mechanisms such as competition or cooperation. The self-replicating nature of IS elements, their interactions with other mobile genetic elements, and the diversity of

host-IS relationships make them ideal candidates for such an ecological perspective. However, despite growing recognition, ecological approaches to ISs have remained largely anecdotal.

In this talk, I aim to fill this gap and demonstrate how a comprehensive transposon ecology framework can provide novel, quantitative insights into the determinants of IS occurrence, both across and within bacterial genomes. To this end, I will present an analysis of IS distribution across 30,482 complete prokaryotic genomes, offering a quantitative ecological characterization of ISs at multiple genomic scales — from their immediate DNA context to their genome-wide organization. I will highlight novel, fundamental genomic features systematically associated with ISs, as well as distinct patterns specific to certain IS families. For instance, I will show that ISs and other mobile genetic elements are ecologically structured within large genomic plasticity regions — up to 100 kbp in length — characterized by coding sequences that are significantly more AT-rich than the rest of the genome. I will also provide evidence for ecological cooperation between ISs, including the ability of ISs to construct ecological niches within genomes that facilitate the insertion and persistence of other IS elements, and likely of horizontally transferred genes as well.



Horizontal gene transfers can reveal ghost lineages

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MEETING ROOM 131, August 20, 2025, 10:30 AM - 12:30 PM

Most of the extant and extinct biodiversity is unknown, but information about these “ghost” lineages may still lie in the genomes of extant ones. Indeed, in addition to the vertical transmission of genetic material from parents to descendants, horizontal transfers (between contemporaneous species) do occur, and this includes transfers coming from these “ghost” lineages. This implies that detecting that a gene has been acquired through horizontal transfer could theoretically give information about unknown lineages.

We have recently demonstrated with simple simulations and empirical Cyanobacteria data that a qualitative signal as to this presence could indeed be observed [1], but further investigation was needed to obtain quantitative results on ghost diversity. To this end, we trained machine learning algorithms to leverage the information about ghost lineages carried by horizontal gene transfer events as detected with phylogenetic methods (with “reconciliation” tools such as ALE, [2]). We first simulated species trees of various sizes, as well as genes evolving in these species and experiencing transfer

events. Second, we subsampled the species to create ghost biodiversity, and third we inferred transfers within these smaller trees with ALE. We then trained neural networks to infer the amount of ghost biodiversity associated with each branch of the sampled species tree.

We observed that gradually increasing the complexity of our machine learning models, from simple multi-layer perceptrons to graph transformers, allowed us to make accurate predictions on a diverse dataset of trees encompassing different transfer rates and numbers of leaves. These results demonstrate the possibility to gain insight into the phylogenetic position and relative abundance of species for which direct evidence of existence (e.g., fossils) is missing.

[1] Tricou et. al. Gene flow can reveal ghost lineages bioRxiv 2024.12.11.627931; doi: <https://doi.org/10.1101/2024.12.11.627931>

[2] Szöllősi et. al. Efficient exploration of the space of reconciled gene trees. Syst Biol. 2013;62(6):901-912.

doi:10.1093/sysbio/syt054



Horizontal transfers of polydnavirus reveals new hosts of parasitoid wasps

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MEETING ROOM 131, August 20, 2025, 10:30 AM - 12:30 PM

Horizontal transfers (HT) of genetic material can be facilitated by close ecological interactions such as parasitism. Polydnaviruses (PDV) of parasitoid wasps are increasingly recognized as a major route of horizontal transfer from wasps to their hosts. PDV are gene delivery agents encoded by domesticated viral genes integrated into the wasp genome. They are injected by female wasps, together with wasp's eggs into host larvae. The circular DNA molecules encapsidated into PDV particles are expressed in host tissues, impairing host immune response and facilitating the development of wasp embryos. Remarkably, these DNA circles integrate massively in the genome of host somatic cells through DNA double strand breaks occurring at a specific locus in the circles called the host integration motif (HIM). We have previously shown that PDV circles have also recurrently entered the germline genome of their hosts through HIM-mediated integration, with traces of ancient HT of multiple PDV circles found in the genome of no less than 124 species from 15 lepidopteran families. Here, we asked whether HT of PDV can be found in non-lepidopteran insects known to be attacked by these parasitoid wasps, as well as in other animal lineages where such

attacks have never been documented. To this end, we used 406 PDV circles from 10 parasitoid wasps as queries to detect HIM-mediated germline integrations of PDVs in 8018 protostomian genomes. In line with earlier findings, our results reveal that such integrations take place not only in Lepidoptera (in 261 species belonging to 32 families) but also in Coleoptera and Hymenoptera (2 and 15 species belonging to 1 and 4 families of, respectively), two orders known to be parasitized by parasitoid wasps. Remarkably, we further detected HIM-mediated HT of PDV circles in four species of Phasmida (stick insects) and one species of Orthoptera (the oak bush-cricket), two orders not previously known to be parasitized by PDV-carrying parasitoid wasps. We find that integrated circles originate from a large diversity of parasitoid wasp lineages, most of which belong to Ichneumonidae, and that they result from recent, species-specific integration events, with only few being shared at orthologous loci between hosts species. Together, these results show that PDV-mediated wasp-to-host transfer of DNA is not limited to lepidopterans and that such events can reveal new ecological interactions.



Introgression recapitulates an ancient hybridisation phenotype in a *Passer* sparrow

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MEETING ROOM 131, August 20, 2025, 10:30 AM - 12:30 PM

Hybridisation and its evolutionary consequences have long fascinated evolutionary biologists. Recent studies leveraging whole genome data have revealed that numerous animal taxa hybridize or show evidence of ancient admixture. Moreover, hybridisation is now increasingly recognized as an important process for forming novel gene combinations and transgressive phenotypes. In addition, there is a long tradition of using hybrid individuals to increase our power to understand the genetic basis of phenotypic traits.

The house, Spanish, and Italian sparrows (*Passer* spp.) are an intriguing system, in which past hybridisation (c. 5000 ya) between the house sparrow (*P. domesticus*) and the Spanish sparrow (*P. hispaniolensis*) has resulted in the formation of a stabilized homoploid hybrid species – the Italian sparrow (*P. italiae*). The Italian sparrow genome is a mosaic of its parents, and the species is phenotypically intermediate between house and Spanish sparrow. The Italian sparrow has a distribution generally restricted to the Italian peninsula and a few Mediterranean islands. However, there are occasional reports of sparrows that, based solely on phenotype, are considered Italian sparrows found far outside their natural range. Furthermore, as well as hybrid species formation, there is evidence of ongoing and more ancient gene flow between house and Spanish sparrows. As such, all European populations of the house sparrow appear to carry some proportion of Spanish sparrow ancestry. Because of this complex history of introgression and hybridization, such vagrant Italian sparrows raise the intriguing possibility that they may

be either first-generation house x Spanish hybrids, backcrossed hybrids, or house sparrows with a de novo mutation of the phenotype or an excess of Spanish ancestry at plumage genes.

Here, we report on the genomic identity of a putatively vagrant Italian sparrow observed in the Netherlands. Using whole-genome sequencing, we compared the genomes of our focal individual to the genomes of individuals sampled from multiple populations of house, Spanish, and Italian sparrow. We unequivocally show that the focal individual is a house sparrow and conclusively rule out the possibility that it is a recent hybrid or backcrossed individual, as well as rule out the possibility of it being an Italian sparrow. We then explore genomic regions that differ between this individual and other *Passer* sparrows to gain insight into genes responsible for the Italian-like phenotype. Our results raise the intriguing possibility that the complex history of introgression in these *Passer* sparrows can lead to an aberrant phenotype, resembling that of an Italian sparrow, within a house sparrow genomic background. Importantly, genomic investigation of such vagrant or aberrant individuals may reveal the phenomenon is more prevalent than currently assumed, and we suggest that molecular identification of vagrant individuals may be needed to make clear species identifications. Furthermore, we suggest that these ‘odd’ individuals have great potential to shed light on genotype-phenotype maps, and we encourage molecular investigations of such individuals be prioritised when vagrants are observed.



Multiple horizontal transfers of immune genes between vertebrates

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MEETING ROOM 131, August 20, 2025, 10:30 AM - 12:30 PM

Background: Horizontal gene transfer (HGT) is less frequent in eukaryotes than in prokaryotes yet can have strong functional implications and was proposed as causal factor for major adaptations in several eukaryotic lineages. HGT detection is difficult to automate in eukaryotes, and problems linked to contamination have long plagued the field. In addition, in many cases, hypotheses alternative to HGT such as incomplete lineage sorting, multiple gene losses, long-distance hybridization and variation in evolutionary rates are difficult to exclude. Despite these limitations, several large-scale studies have reported hundreds of solid cases of HGT in multiple eukaryotic lineages such as microbial eukaryotes, plants, fungi and insects. However, most of these studies tended to focus on inter-domain HGT rather than within-eukaryote transfers, because the power to infer HGT increases with the phylogenetic distance between donor and receiving lineages. To start filling this gap, we performed a systematic search for HGT events within two vertebrate lineages, ray-finned fishes and amniotes.

Materials and methods: We developed a semi-automated and multipronged approach to detect HGT among 242 fishes and among 616 amniotes. First, we postulated that transferred genes should show a lower divergence than most vertically transmitted genes. Thus, for each possible pair of species, we computed pairwise dS values between all orthologous genes, and selected genes characterized by extremely low dS values (below the 0.5% quantile of the distribution). We then reasoned that it would be highly unlikely for a transferred gene to be located at the same locus, i.e., between the same orthologous genes in the donor and recipient species. Thus, for each retained gene (based on low dS values), we computed a microsyntenic score, representing the number of identical genes among flanking genes between the two species. Only genes with a null

microsyntenic score (no identical genes among the flanking genes) were retained. Finally, we only retained candidates for which gene phylogenies, computed from multiple independent alignment and trimming methods, were congruent with a HGT scenario, i.e., trees in which genes from paraphyletic species in the species phylogeny form a robust monophyletic group.

Results: We identified multiple teleost-to-teleost, as well as amniote-to-amniote HGT events. All transferred genes were found in multiple independent genomes from the donor and recipient clades, ruling out the alternative contamination hypothesis. Manual curation revealed that in most instances, there was a high nucleotide identity, between donor and recipient lineages, which extended to intronic and/or flanking sequences, which sometimes even included shared TEs (likely co-transferred with the gene). The distribution of HGT events in the species tree was highly heterogeneous, with most transfers occurring between the same species clades. Among HGTs identified, we retrieved the only transfer reported to date between vertebrates, of an antifreeze protein between Clupeiformes and Osmeriformes. Besides this case, we found that most transferred genes play roles in immunity, suggesting that they may be more likely than others to confer strong selective advantage to the recipient species. While we did not find any significant changes in the evolutionary rate of genes upon reception, we found that transfers are often followed by duplications, which might have contributed to expanding the newly acquired beneficial functional gain.

Conclusion: We report, for the first time, multiple horizontal gene transfers between vertebrates which, although rare (around thirty), may have contributed to the evolution and adaptation of their immune systems.





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S36 - Microevolutionary processes and Macroevolutionary patterns



A Ghost in the Stream: Mitochondrial Evidence for a Cryptic Lamprey Lineage in the Upper Po Valley

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POSTER SESSION 2 - **P02.314**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Lampreys of the genus *Lampetra* are evolutionarily significant representatives of jawless vertebrates (Agnatha) and a key focus in studies of early vertebrate diversification. The early evolutionary history of *Lampetra* in the Adriatic basin occurred in the Plio-Pleistocene (~2.9 MYA), after the Messinian Salinity Crisis (MSC). *Lampetra zanandreae*, originally described from northeastern Italy, has a patchy and discontinuous distribution across the Alpine drainages to the Adriatic slope of central Italy. Recent work by Rüber et al. (2023) suggested that populations historically identified as *L. zanandreae* in the northwestern Po River basin may constitute a genetically distinct and undescribed lineage, potentially a separate species. This region's complex paleo-hydrological history, shaped by Pleistocene glaciation and the formation of fluvioglacial megafans, likely contributed to population isolation and divergence. However, these findings were based solely on a fragment of the COI gene, leaving the phylogenetic placement and mitochondrial differentiation of these populations unresolved.

Materials and Methods: To investigate mitochondrial genome divergence in *Lampetra*, we extracted total DNA from population samples representing various Italian river basins, including the Po Valley, Le Marche and Piemonte region. DNA was obtained from whole-genome resequencing (DNBseq short-read data), and mitochondrial genomes were de novo assembled using MitoZ (v3.5). A phylogenetic dataset was constructed using the nucleotide sequences of the 13 protein-

coding genes (PCGs), aligned by codons. These sequences were concatenated into a supermatrix and analyzed using maximum likelihood (IQ-TREE v2.3.3), with partitioning schemes optimized by PartitionFinder (v2.1.1). We compared newly assembled mitochondrial genomes to 26 publicly available agnathan (*Petromyzontiformes* and *Myxiniiformes*) mitogenomes and one outgroup, *Zenarchopterus buffonis*.

Results: Our mitochondrial phylogeny confirms the findings of Rüber et al. (2023): the population historically labeled as *Lampetra zanandreae* (*L. 'upper Po'*) from the Piemonte region is genetically distinct from other *L. zanandreae* lineages present in Italy. In fact, *L. 'upper Po'* clusters more closely with *L. fluviatilis* and *L. planerii*. These results suggest the presence of a previously unrecognized lineage within the upper Po River, supporting the hypothesis of a cryptic species that diverged in historical isolation due to complex Pliocene and Pleistocene drainage evolution in the region.

Conclusions: Our results reinforce the need for taxonomic re-evaluation of northwestern Italian *Lampetra* populations. Mitochondrial genome data provide robust evidence for the existence of a highly divergent lineage in the upper Po Valley, likely shaped by the area's unique biogeographic history and paleo-drainage fragmentation. This work underscores the value of mitogenomic approaches in identifying cryptic diversity and informs ongoing efforts in lamprey systematics, evolutionary biology, and conservation planning.



Adaptation to whole genome duplication across scales : convergent evolution among Angiosperm plant species

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POSTER SESSION 2 - **P02.300**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

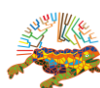
Polyploidisation brings one of the most remarkable paradoxes in evolution. While being putatively the largest deleterious mutation an organism can encounter, polyploids are widespread in nature and often successful. Despite decades of theoretical research and phenotypic characterization of this paradox, very little is known about the genetic basis and repeatability of adaptation to WGD across different natural WGD transitions.

Autotetraploidization, further referred to as whole-genome duplication (WGD) is always simply the doubling of the DNA content in the cellular environment, while the more studied allopolyploidisation includes the confounding effect of hybridization. WGD pervasiveness across plant taxa constitutes a fantastic natural experiment to evaluate the extent to which evolution is repeatable, by studying a consistent challenge across species. A common hypothesis is that WGD requires a set of functional adaptations that might be shared between species. To date, only three autotetraploid lineages, all from a single family (Brassicaceae), have been the purpose of genomic analyses to adaptation to WGD, involving comparison between naturally stable autotetraploid lineages and their diploid sisters, namely *Arabidopsis arenosa*, *Cardamine amara* and *Cochleria* spp. Those pioneering studies have uncovered several mechanisms under selection, that were repeatedly found across species. As theoretical works predicted, genes and functions involved in the stabilisation of meiosis are consistently under selection. Genome scans of selection further identified unexpected importance of pollen tube growth. Together, these isolated case studies suggest that there are shared

mechanisms for adaptation to WGD. To which extent evolution repeats itself at the levels of genes and molecular functions, however, needs further systematic quantification using uniform methodology across a broader set of species that recently adapted to WGD.

In this ongoing project, I am combining novel comparative population genomic techniques and cytogenetics across twelve natural polyploid plant species distributed across the Angiosperm phylogeny. I assembled a pipeline to perform systematic genome scan for selection by contrasting conspecific diploid and recently evolved autotetraploid populations. Leveraging a unique short-read sequence dataset through this unified method, I aim at revealing the general mechanisms of WGD adaptation and study the determinant of convergent adaptation from the population scale to a wide array of plant species.

Preliminary results from the study of *Alyssum montanum* suggested a significant overlap between now four species, including the meiosis gene *ASY3*, the transcription initiation factor *IIF*, and the ion homeostatic gene *HKT1*, pointing out the necessity to regulate meiosis and homeostasis for cell division and stability after WGD. By combining pairwise gene set overlap and phylogenetic data, those preliminary results also suggest that the extent to which species share functional adaptation and genetic convergence might depend on the divergence time between species. This work will significantly improve our understanding of how plants handle WGD, including crops, and will be a milestone to the fundamental question of the repeatability in evolution.



Assessing the population history of crop infecting pathogenic fungi and their relatives infecting wild grasses

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POSTER SESSION 2 - **P02.310**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Fungal plant pathogens are components of all ecosystems and a permanent concern in agriculture. Apprehending their evolutionary history is crucial to determining the origin of existing crop pathogens and predicting the emergence of new ones. Evolutionary statistical genetics offers an opportunity to reconstruct population histories based on whole-genome data of closely related fungal species. One statistic that can be assessed from comparative sequence analyses is the extent of Incomplete Lineage Sorting (ILS) among closely related species. Incomplete Lineage Sorting is a phenomenon that results from maintained polymorphisms in ancestral species. It is evidenced in genomes when local gene trees are incongruent with the species tree. The amount of ILS between related species depends only on the characteristics of their ancestors, in particular on ancestral population size and relative speciation times. In this study, we addressed the evolutionary history of the wheat-infecting fungal pathogen *Zymoseptoria tritici* using genome data from a set of closely related species. We quantified the extent of ILS between *Z. tritici* and its closely related species using high-quality genomes and a new method implemented in the TRAILS program. Using this approach, we

determined population characteristics in ancestral species within the *Zymoseptoria* genus. We confirmed estimates obtained with a smaller genome dataset and extended these results, showing that ILS is widespread in the genus. Overall, we highlight that speciation did not involve population bottlenecks, but rather occurred in diverging lineages with high effective population sizes between 10,000 and 50,000 generations ago. In addition, we observed a high variability in ILS along the genome, both along individual chromosomes and among chromosomes of different lengths. Shorter chromosomes and chromosome ends have a higher extent of ILS. This could be the consequence of a larger effective population size in these regions, where a higher recombination rate would unlink selected and unselected sites more effectively. In conclusion, our study used genomic data to uncover the rapid evolution and high speciation rates within the *Zymoseptoria* genus. These important plant pathogenic fungi arose from large ancestral populations. Speciation of the wheat pathogen likely coincided with crop domestication. By investigating local variation of ILS, we might identify genes under selection pressure in ancestors that participated in species divergences.



Comparative establishment of species barriers in plants and animals by measuring introgression among species pairs from phylogenies

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POSTER SESSION 2 - **P02.294**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Speciation, the process through which new reproductively isolated species emerge from ancestral populations, occurs due to the gradual accumulation of barriers to gene flow within genomes. The accumulation of such microevolutionary barriers progressively increases the reproductive isolation between diverging lineages. As divergence starts gene flow only occurs in genomic regions that are not linked to species barriers, so that the diverging lineages can be considered as two semi-isolated species. Speciation is completed when all genomic positions are linked to species barriers.

Hybridization and introgression are historically believed to be more prevalent in plants than in animals. However, recent findings from our team, employing population genomics approaches, demonstrate that, contrary to this view,

reproductive barriers establish more rapidly in plants than in animals at equivalent levels of genetic differentiation. Whereas this previous study focused on contemporary gene flow, we now extend our investigations by exploring whether the observed differential dynamics between plants and animals are also historical and identifiable from the phylogenetic histories of clades within these kingdoms. Specifically, we undertake comparative studies of the dynamics of introgression among plants and animals over deep macroevolutionary time, using phylogenomic approaches based on 100s or 1000s of loci. Currently we have analysed 14704 pairs of plant species and 38744 pairs of animal species. Our preliminary results indicate that on average genetic exchanges among animal species persist at higher levels of divergence than they do among plant species, even at deeper macroevolutionary scales.



Cryptic species complex in a Korean endemic freshwater fish: genetic structure and morphological analyses of the Korean dark chub (*Nipponocypris koreanus*) compared with its close relative (*Nipponocypris temminckii*)

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POSTER SESSION 2 - **P02.283**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Allopatric speciation has long been considered as a primary mechanism for the origin of species in freshwater ecosystems as geographically disconnected rivers can serve as a 'physical barrier' to gene flow among populations. Geographic isolation by physical barriers would restrict gene flow across populations, driving inter-population genetic differentiation. This could lead to ecotype formation and even allopatric speciation through adaptations to different environmental conditions by divergent natural selection. Geological/glacial activities in the past shaped major river basins in the Korean Peninsula. Geographical populations of freshwater fishes among different river drainages have evolved a number of endemic species. There are 213 freshwater fishes identified in South Korea, of which 67 (approximately 32%) are indigenous species. *Nipponocypris koreanus*, a widely distributed Korean endemic species, was recently classified as a separate species from its relative *Nipponocypris temminckii*. However, morphological differences between the two species are ambiguous and genetic differences between and within species remain largely unexplored. In this study, using mitochondrial DNA cytochrome oxidase I (COI), control region (CR) and our developed nine microsatellite loci, we aimed to determine the phylogenetic relationships and population genetic structure among geographically separated six river basins across its nearly entire distributional ranges. We further attempted to genetically verify the species boundary (e.g., species complex) between *N. koreanus* and *N. temminckii* using whole mitogenomes.

We collected a total of 829 individuals of *N. koreanus* [mtDNA (N = 727), microsatellites (N = 717)] from 28 different localities within six river basins [the Han River (HR; number of populations, N = 12), East-flowing river (ER; N = 4), Geum River (GR; N = 3), Nakdong River (NR; N = 7), Seomjin River (SR; N = 1) and Mangyeong River (MR; N=1)] in

South Korea. Thirty individuals of *N. temminckii* [mtDNA (N = 27), microsatellites (N = 30)] were sampled at only one site from the Geum River. We analyzed phylogenetic relationships by using concatenated mtDNA COI (625 bp) and CR (832 bp) sequences. We also analyzed the population genetic structure of *N. koreanus* and *N. temminckii* using nine newly developed microsatellite markers. Furthermore, we analyzed the mitogenomes of the *N. koreanus* populations with *N. temminckii* to quantify their level of genetic divergence.

We found that *N. koreanus* evolved relatively well-separated clades in each river basin, and that some morphologically *N. koreanus* individuals were genetically *N. temminckii*, suggesting the possibility of hybridization between the species or phenotypic plasticity with similar morphology evolved in the species occurring in the same environments. *Nipponocypris koreanus* individuals from the Nakdong and Seomjin Rivers are the most closely related to *N. temminckii*, and they probably represent cryptic species complex as they showed a genetic distance of 2.2~2.9%, although *N. koreanus* had a distinct genetic structure. The results of comparing the mitogenomes from different rivers also showed that the Nakdong and Seomjin River populations had a genetic distance of 4.7~5.1%, further supporting the notion that *N. koreanus* is a cryptic species rather than a single species.

The results of the study suggest that *N. koreanus*, a widely distributed freshwater fish across South Korea, evolved riverine unique genetic lineages and structures, particularly for the Nakdong and Seomjin Rivers, which house their own genetic integrities. Our findings will also shed an intriguing insight into understanding how Korean freshwater fishes have evolved in response to geographically isolated major river/stream environments.



Detecting evolutionary shifts in primates through model-based comparative analyses.

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POSTER SESSION 2 - **P02.289**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

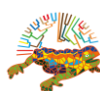
Evolutionary shifts—large deviations in phenotypic values between species—can reveal important episodes of convergence and divergence in trait evolution, yet they are not explicitly modeled in most standard comparative frameworks. Typically, stochastic evolutionary models such as Brownian Motion (BM), Ornstein–Uhlenbeck (OU), or acceleration–deceleration (AC/DC) are used to describe trait evolution under neutrality, but they do not directly address where or when shifts occur. Addressing shifts is important to obtain accurate evolutionary inferences, particularly if multiple shifts have occurred during trait evolution.

In this study, we present PhyloQ, a model-aware statistical test designed to detect such shifts by comparing observed phenotypic differences between species to trait-specific null distributions generated under the best-fitting evolutionary process for each trait. We applied this approach to 77 continuous traits in primates—including life-history (e.g., maximum lifespan), metabolic, and morphological traits (e.g., body mass, brain size)—using the high-resolution phylogeny from Kuderna et al. (2023). For each trait, we first identified the most likely underlying evolutionary process (BM, OU, or Early Burst), then used PhyloQ to generate the corresponding null expectations and detect significant shifts across the primate tree. Compared to existing methods (Felsenstein, 1985; Garland et al., 1992; Stayton, 2015; Castiglione et al., 2019), PhyloQ offers a more flexible and intuitive framework that accommodates trait-specific evolutionary dynamics and allows for the detection of both convergent and divergent patterns.

Our findings confirm our hypothesis on the impact of evolutionary process selection on evolutionary inference and demonstrate how model-based simulations can enhance the resolution of macroevolutionary analyses. Our approach provides a flexible, model-aware framework for detecting evolutionary shifts, offering new insights into the dynamics of convergence and divergence across species.

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Developing an experimental toolbox for entomopathogenic nematodes

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POSTER SESSION 2 - **P02.287**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Entomopathogenic nematodes (EPNs) are microscopic soil dwelling pathogens that cause microbial symbiont-aided lethal infections in insect larvae. They are phylogenetically closely related to free-living and parasitic nematodes, and their life cycles share similarities. Specifically, the EPN infective juvenile (IJ) stage is reminiscent of *Caenorhabditis elegans* dauer larvae and infective larvae of helminths. This life stage is resilient to environmental stressors and has previously been suggested to be an essential stage in the evolution of parasitism. EPNs are a promising biocontrol agent, as they naturally regulate insect populations, but have only proven effective in biocontrol at small scales. To optimize their use at an agricultural scale, it is imperative that we better understand their biology, from infection efficiency and prey preference to resistance against environmental stressors. To date, high-throughput workflows for characterising relevant EPN phenotypes and their genomic basis are lacking. Our work focuses on the development of assays to phenotype and genetically characterize EPNs on a large scale, and to generate genomic resources. These tools and resources can realise the implementation of this tri-factorial system as a standardized model system to study traits relevant to biocontrol optimization and host-pathogen interactions. Here we present our initial steps to establishing this model system.

Methods: *Drosophila melanogaster* at various larval stages were infected with one of three concentrations of EPNs (*Steinernema feltiae* and *Heterorhabditis bacteriophora*) and their development was tracked until three days after emergence of adult flies. Larvae to

pupae and pupae to adult fly survival was recorded. Larvae and pupae with visible EPN infections were collected to harvest hatching IJs. We optimized a metabarcoding approach to identify EPNs after soil-sampling, amplifying a fragment spanning the internal spacer regions of the ribosomal rRNA genes. Results: At identical EPN:prey ratios *S. feltiae* had a significantly higher infection efficiency compared to *H. bacteriophora*. A ratio of 50:1 *S. feltiae*:*D. melanogaster* yielded a similar infection rate to 500:1 *H. bacteriophora*:*D. melanogaster*. Infected first and second instar larvae mostly died before pupating, while third instar larvae pupated but did not hatch. With the optimal infection ratio and *Drosophila* larval stage, we are currently testing the effect of EPN age and short term UV exposure on infection efficiency. With the optimized metabarcoding approach we are identifying local EPNs collected around various agricultural lands in Denmark.

Conclusions: The development of a toolbox to characterize EPN phenotypes and their genomic basis will facilitate optimization of EPNs as a biocontrol agent and as a model to study the evolution of parasitism. Future applications of optimizing this tri-factorial system to study host-pathogen interactions include the use of *C. elegans* to test functional hypotheses, akin to the synergy between studies in parasitic nematodes and *C. elegans*. And at a wider scale, comparative genomics of EPNs with both free-living and parasitic nematodes can help elucidate genes or pathways of interest for various scientific questions, especially those relating to traits relevant for biocontrol optimization and the identification of novel nematode-infection mitigation strategies.



Disentangling effective population size and mutation rate: amphioxus at the edge of panmixia

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POSTER SESSION 2 - **P02.292**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Genomic diversity, or how the genomes of individuals of the same species differ from each other, varies widely among species and plays a major role in their adaptability. Disentangling the causes behind genomic diversity at the population scale is essential to understanding the evolutionary capacity and survival of any species at longer time scales. Here we address the case of a slowly evolving and phenotypically conserved species which is genomically extremely diverse: the marine chordate amphioxus, in particular the European species (*Branchiostoma lanceolatum*). We confidently estimate that around 3% of sites in the genome of each European amphioxus individual are heterozygous in samples from two distant wild populations (Mediterranean and Atlantic), while confirming short phylogenetic branches leading to amphioxus among chordates. The Mediterranean and

the Atlantic populations are distinct but closely related despite their geographic distance. Both contrasts between macro-evolutionary and population patterns, and simulations show that the high genomic diversity of amphioxus is consistent with a high effective population size rather than a high mutation rate. Overall, our results support that a long-standing high effective population size is responsible for the patterns seen at a larger evolutionary scale in amphioxus (i.e. enormous genomic diversity, slow substitution rate and slow phenotypic evolution). This study highlights the exceptionality of amphioxus' evolution and contributes to the understanding of the relationship between genomic properties at the population and species levels and timescales.



Eco-evolutionary processes can explain morphological similarities in cryptic species complexes

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POSTER SESSION 2 - **P02.273**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Cryptic species (CS) are reproductively isolated units that are morphologically indistinguishable, which have been increasingly identified in the marine environment with the rise of molecular markers. Overlooking or ignoring CS can mean misestimating ecological parameters, including estimates of biodiversity or connectivity. CS may also be able to tell us about the link between small-scale processes and large-scale patterns of morphological differentiation among species. We conducted a literature review and identified over 900 CS complexes in free-living marine animal species. We analyzed this dataset to test both human and eco-evolutionary processes of CS formation. We focused on distinguishing species displaying diagnostic differences (“pseudo-CS”) from those that were CS *sensu stricto* (CSss). Using generalized linear mixed models, we

found poor support for history of science variables in predicting if a species is CSss. Species living in soft sediment were more likely to be CSss, and CS in sympatry were more likely to be pseudo-CS. These results support the role of stabilizing selection in the maintenance of CS (in soft sediment) and of divergent selection in increasing morphological divergence among sympatric species. CS in widespread and connected habitats were more likely to show statistical (but not diagnostic) differences, supporting the idea that large effective population sizes can delay fixation of morphological differences as under neutral morphological evolution. Overall, our results show the importance of eco-evolutionary processes in CS formation: CS are not simply cases of human error, but can be caused by biological phenomena.



Effects of cycles of range expansion and contraction on macroevolutionary patterns

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POSTER SESSION 2 - **P02.303**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: The Quaternary was marked by glacial cycles and climatic fluctuations that led to expansions and contractions of different biomes, affecting the local species diversity and population structure of various groups. These shifts have promoted the emergence of isolated refugia—fragmented biomes surrounded by another larger biome—that have long been hypothesized to have promoted species diversification by driving population isolation and allopatric speciation. Here, we implemented spatially explicit individual-based simulations using the Derrida-Higgs neutral model of speciation. This approach allowed us to model macroevolutionary outcomes of biome contraction and expansion, including the formation of refugia, reflecting Quaternary landscape fluctuations.

Material and Methods: Each simulation included two cycles of contraction and expansion, with initial conditions starting either in a contracted or expanded state. During contraction phases, the landscape could form a single continuous area (one refugium) or separated areas (two or four isolated refugia). For the scenarios with multiple refugia, geographical barriers were placed, either fixed or varying in location among cycles. We also conducted control simulations with areas that were either constantly contracted or expanded. We manipulated the reproductive genetic threshold (G) so that in one scenario, sympatric speciation is not possible in the expanded area (lower G), and in another, in which sympatric speciation is possible in the expanded area but not in the contracted area (higher G). From the simulation results, we constructed phylogenetic trees by recording the genealogies for the entire population and recording the time of origination of the Most Recent Common Ancestor (MRCA) between each pair of individuals. These were used to calculate diversification rates over time and the balance of the trees. We then derived net diversification rates, tree balance metrics, and total species counts for each scenario.

Results: Contraction dynamics only drove speciation in the scenarios with low reproductive threshold (lower G), where the barriers were necessary for the formation of species. The number of species originating in the scenario with two barriers led to more speciation if the location of the barriers varied through time. However, each subsequent expansion phase reduced species numbers due to merging populations. In the scenarios with a higher genetic threshold (higher G), where sympatric speciation could occur, area size was the main determinant of species richness through time, with the presence of barriers not affecting the maximum number of species. The highest number of species produced with lower G was equal to or fewer than the number of species in simulations with a constantly expanding area. Notably, these simulations often produced more species than the lower G simulations with barriers. Refugia consistently led to more balanced phylogenetic trees when simulations began in a contracted state. Across all simulations, diversification rates increased through time, with noticeable shifts in slope corresponding to landscape expansion (acceleration) or contraction (deceleration).

Conclusions: The neutral landscape dynamics of contraction and expansion with barriers led to increased diversification only when sympatric speciation was not possible. When sympatric speciation was allowed, area size (not landscape dynamics), was the main factor determining speciation. In all simulations, allopatry influenced the balance of the resulting phylogenies when the landscape started in a contracted state. Overall, our simulations show that the landscape dynamics with barriers led to an increase in diversification only when sympatric speciation would be unlikely.



Evolution of nest architecture in tyrant flycatchers and allies

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POSTER SESSION 2 - P02.291, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Innovations in nest design are thought to be a key factor in the evolutionary success of passerine birds (order: Passeriformes), which colonized new ecological niches as they diversified during the Oligocene and Miocene. In particular, tyrant flycatchers and their allies (parvorder: Tyrannida) are an extremely diverse group of New World suboscine passerines that occupy a wide range of habitats and exhibit substantial variation in nest design.

To explore the evolution of nest architecture in this clade, we first described nest traits across the Tyrannida phylogeny and estimated ancestral nest conditions. We then quantified macroevolutionary transition rates between nest types, examined potential coevolution between nest type and

habitat, and used phylogenetic mixed models to identify possible ecological and environmental correlates of nest design.

Our results suggest that the Tyrannida ancestor most likely built a cup nest in a closed habitat, and that dome nests evolved independently at least 15 times within the group. Both cup- and dome-nesting species diversified into semi-open and open habitats, and we found no evidence for coevolution between nest type and habitat. Moreover, nest type was not significantly associated with several key ecological, life-history, or environmental traits, suggesting that the broad variation in Tyrannida nest architecture may not be driven by any single factor.



Evolutionary patterns of tolerance to defoliation in the genus *Datura* (Solanaceae)

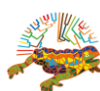
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POSTER SESSION 2 - **P02.277**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Plants defend themselves from herbivores, but herbivores keep consuming plant tissues despite the wide array of physical and chemical resistance traits that plants possess. However, plants can buffer the negative effects of herbivory on fitness by tolerance. Thus, tolerance is an important strategy for plants to cope with herbivory. How tolerant responses evolved, and which are the underlying traits that influence them remains an important topic in evolutionary ecology. Here, we address the evolution of tolerance to leaf damage in the genus *Datura*, a group of self-compatible plants, native to arid and semiarid environments in Mexico and southern USA, through a common garden experiment with simulated herbivory damage. We raised ten of the ca. thirteen species of the genus in pots with the same soil (a 3:2 mix of sand and perlite) in a greenhouse. We randomly allocated plants into two groups, undamaged (control) and damaged (simulated herbivory). Simulated herbivory was applied when plants reached its reproductive stage, mimicking the timing and pattern of leaf damage caused by herbivores. Tolerance to leaf damage and traits potentially associated with tolerance were measured (foliar and biomass allocation traits). Analyses were conducted within the phylogenetic comparative method framework, evaluating phylogenetic signal (Blomberg's K) and phylogenetic correlations (PGLS) between measured traits and

tolerance. We also examined the relationship between plant chemical/physical defenses (resistance) and tolerance and the relationship between fitness (seed production) in absence of damage and tolerance to assess potential constraints on evolution of tolerance. Results indicate no significant phylogenetic signal for tolerance. Biomass allocation to roots and leaves (RMF and LMF, respectively), specific leaf area (SLA) and chlorophyll content (CCI), are significantly correlated with tolerance. Both SLA and CCI showed significant phylogenetic signal was detected. No trade-off between resistance and tolerance across species was detected. Instead, we found a positive relationship between tolerance and two of the main chemical defenses of *Datura*. A significant negative relationship between tolerance and fitness in absence of damage was found. The results suggest that tolerance has evolved independently of the evolutionary relationships between *Datura* species but the variation in underlying or correlated traits with tolerance are influenced by the phylogeny, specifically the foliar traits. While the species resistance levels do not trade-off with tolerance in *Datura*, the detected negative relationship between tolerance and seed production in absence of damage may constraint the evolution of tolerance in this genus.



Experimental evolution of metabolic fluxes in a nutritionally challenging context

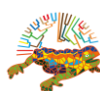
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POSTER SESSION 2 - **P02.279**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Metabolic differences among and within species are pervasive and are interpreted not only as plasticity, but also as adaptive components of the phenotypes. The metabolic machinery is expected to work in accordance with the availability of energy and chemical building blocks in the environment to bring about relevant growth and reproduction. As such, metabolism is at the heart of evolutionary adaptation to new environments. The level of central metabolism metabolites can only tell how much about the dynamic functioning of the organism as it indicates the level of activity of a pathway. However, combining isotopic methods, high precision mass spectrometry, and time-framed experiments can give actual quantitative information about the fluxes of single metabolites a the whole organism level. We used experimentally

evolved population of *Drosophila melanogaster* submitted to poor food condition for more than 350 generations to ask how much the fluxes of amino acids, so crucial for growth, differed from control populations in common garden experiments. Our results indicate that in poor food conditions, most amino acids fluxes increased in the populations evolved on poor food compared to the control populations maintained on standard food, which may contribute to their higher fitness in this challenging environment. Our results indicate that the metabolic machinery responsible for protein and amino acid digestion, absorption, transport and usage evolved as to confer faster growth to populations maintained on a challenging food medium.



Extreme Morphological Divergence Without Visual Compromise: Conserved Visual Field Geometry in Sexually Dimorphic Spiders

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POSTER SESSION 2 - **P02.276**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Males across numerous animals exhibit exaggerated traits, often highly conspicuous—large in size, and/or vivid in coloration—that serve to increase their reproductive success by attracting mates or deterring rivals. While the benefits of these sexually selected structures in terms of mating advantages are well documented, their potential impact on functional traits remains poorly understood. These structures may increase predation risk and energetic expenditure, and thereby reduce longevity, and impair function in critical organ systems. These costs are central to understanding the evolutionary trade-offs that shape sexual dimorphism. Despite their prominence, quantification of such functional trade-offs has been limited, leaving critical gaps in our knowledge of how natural and sexual selection interact. Addressing this issue is essential for unraveling one of the most intriguing puzzles in evolutionary biology: why such costly traits persist across diverse taxa. In erigonine spiders, males frequently exhibit extreme and elaborate cephalothorax modifications that appear to dramatically alter the positioning and orientation of the eyes and, thus, the function of the visual system. We investigated whether these putative sexually selected traits come at a cost to visual system performance in males.

Materials and methods: To assess potential sex-specific differences in visual system morphology and function, we applied three-dimensional geometric morphometrics and optical modeling to both males and females across 10 erigonine species. We quantified and compared eye arrangement, visual system diversity, and the size and orientation of visual fields between the sexes.

Results: Our results reveal significant and pronounced differences between males and females concerning carapace shape, eye position and orientation, and the diversity of the visual system, as quantified by morphological disparity in eye arrangements. Remarkably, however, males maintain visual fields that are comparable to those of females in terms of size, orientation, and binocular overlap, despite these substantial morphological divergences.

Conclusions: Our findings underscore the selective pressures on male visual systems to maintain visual field characteristics comparable to those of females, despite pronounced morphological divergence. We conclude that vision is a key sensory modality in these enigmatic animals. More broadly, our results demonstrate that male fitness in sexually dimorphic species is influenced synergistically by both natural and sexual selection.



Flower traits and their relationship with evolution of tolerance to herbivory in *Datura* plants

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POSTER SESSION 2 - P02.293, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Tolerance, jointly with resistance, are the main strategies of plants to deal with herbivory. It is expected that plant tolerance to herbivory evolves when plants experience damage despite its resistance level (this is possible when a specialist herbivore consumes the plant). Thus, how often a plant species receive herbivory damage must be positively correlated with its tolerance level. Leaves of *Datura* plants (Solanaceae) are consumed by generalist and specialist herbivores. Specialist herbivores can defoliate plants completely, some of which are also pollinators of these plants, specifically, moths from the Sphingidae family. *Datura* flowers can be very conspicuous, white to purple colored, trumpet or funnel shaped flowers and there is significant variation in size between species. Previous studies have found that preference for moths on *Datura* depends on flower quantity, flower size, and nectar volume and quality. Given that when pollination occurs, female moths oviposit and the caterpillar can defoliate completely a plant, pollination attraction can be a good index of the herbivory a *Datura* plant can receive. Since reproduction and herbivory are likely to be linked in this system, we explored if plants' pollinator attractiveness is correlated with tolerance to herbivory. We

grew ten species of *datura* in a common garden setting to reduce environmental influence in our flower measurements. Measurements were consistent with previous reports. We measured pollinator attractiveness as floral traits. Flower size (length and mass), nectar quantity (volume) and quality (sugar content as brix degrees), and relative biomass allocation to flower structures (corolla, calyx, feminine and masculine parts) were measured. Then its relationship with tolerance to herbivory was assessed by phylogenetic comparative methods (PGLS). Floral trait variation was summarized using phylogenetic PCA. The main axis of variation (phyPC1) represented the general flower size. Traits related to flower size, quality of nectar and biomass allocated to male function were positively correlated with tolerance. These traits commonly characterize plants attractive to pollinators. There was not a phylogenetical structure of variation on flower size. The pattern found here suggests that in *Datura*, plant tolerance to herbivory has evolved jointly with attractive flowers to Sphingidae moths, as a likely product of the dual interaction of these moths, as pollinators and herbivores. This pattern also suggests that ecological processes may be driving the variation on tolerance to herbivory in *Datura*.



Fossil Placement in Dated Phylogenies: A Case Study on Bark Beetles (Coleoptera: Curculionidae)

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POSTER SESSION 2 - **P02.270**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Understanding the evolutionary history of clades requires robust divergence time estimates, yet this process is often hindered by uncertainties in fossil placement. The evolution of bark beetles (Scolytinae) has long been linked to the radiation of angiosperms, with their diversification assumed to have occurred in parallel with the expansion of flowering plants. However, the timing and ecological context of their early evolution remain debated. The oldest unambiguous scolytine fossil, †*Cylindrobrotus pectinatus*, from Lebanese amber (125.77–121.4 Ma), has traditionally been used to calibrate the entire clade, despite its phylogenetic position never being formally tested. This assumption has contributed to uncertainty regarding the true timing of Scolytinae diversification. Here, we refined the evolutionary timeline of Scolytinae by applying total-evidence dating, integrating morphological and molecular data along with 15 well-preserved fossils as terminal taxa. We also employed traditional node dating and the Bayesian Brownian Bridge model to independently analyse the fossil record of Scolytinae, cross-validating our results. By incorporating multiple methodologies, we aimed to mitigate biases associated with any single dating approach and achieve more reliable divergence time estimates. Our results indicate that all three methods produced consistent outcomes,

supporting a Late Jurassic to Early Cretaceous origin for Scolytinae (ca. 160–135 Ma), earlier than previously assumed. This revised timeline suggests that early scolytines may have initially evolved in gymnosperm-dominated ecosystems before later adapting to angiosperms as they became more dominant. Additionally, our analyses challenge the traditional placement of †*Cylindrobrotus pectinatus* as a stem lineage of Scolytinae. Instead, our results suggest that this fossil is more closely related to clades nested within Scolytinae. As †*Cylindrobrotus pectinatus* is found inside Scolytinae rather than being a stem lineage, previous estimates of scolytine divergence that relied on this fossil as a calibration point may have underestimated the age of the clade. By implementing multiple dating methodologies, our study underscores the importance of fossil placement in reconstructing evolutionary histories and demonstrates that, when correctly parameterized, different approaches yield congruent results. While here Scolytinae serve as a case study, the methodological framework we applied is widely applicable to other clades. Moreover, these findings contribute to a broader effort to refine evolutionary timelines and improve the robustness of divergence estimates across different clades.



From microevolution to macroevolution: sexual selection in plants shapes speciation in a 1000-species phylogeny of Brassicaceae

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POSTER SESSION 2 - **P02.278**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

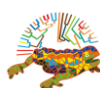
Background: Sexual selection is a well-established driver of trait evolution in animals, but its role in speciation remains debated. While some studies suggest that sexual selection accelerates speciation by fostering reproductive isolation, others find neutral or even negative effects. In plants, sexual selection has gained increasing attention, operating both before pollination (via competition for pollinators) and after pollination (via pollen competition and female choice mediated by pistil traits). However, whether sexual selection promotes speciation in plants is still unresolved. We hypothesize that clades experiencing stronger sexual selection should exhibit higher speciation rates due to faster reproductive barrier evolution. **Materials and methods:** We examined the relationship between sexual selection proxies and speciation rates in Brassicaceae. Petal size (length and width) and pollen-to-ovule (P: O) ratios served as proxies for pre-pollination sexual selection, while style length represented post-pollination sexual selection. Trait data were compiled from direct measurements, literature sources, and machine learning-based imputations. Mating system and ploidy data were similarly assembled to control for confounding effects.

Phylogenetic analyses were based on a 1004-species Brassicaceae tree (eight outgroups), constructed using maximum likelihood from nuclear ribosomal and chloroplast DNA sequences, and time-calibrated in BEAST2. We first tested whether mating system and ploidy explained variation in sexual selection proxies. Diversification analyses included Quantitative

State Speciation and Extinction (QuaSSE) models for trait-dependent speciation, Tip-Rate Correlation (TRC) tests with λ DR (validated by ES-sim), and phylogenetic generalized least squares (PGLS) regressions for λ BAMM rates. Hidden-State Speciation and Extinction (HiSSE) models were used to test for independent effects of mating system and ploidy on diversification.

Results: Mating system explained significant variation of all sexual selection proxies, while ploidy had no effect. HiSSE models showed that selfing lineages diversified more slowly than outcrossing ones, with no impact of ploidy. QuaSSE analyses revealed a robust hump-shaped relationship between sexual selection and speciation, even after controlling for mating system effect. A weak association between sexual selection proxies and λ DR was observed, but ES-sim analyses indicated it was not statistically significant. Similarly, PGLS analyses found no significant relationship between sexual selection proxies and λ BAMM tip speciation rates.

Conclusions: These results partially support the hypothesis that sexual selection promotes speciation in plants, and were dependent on the method used. Although robustness checks (Type I error control and ES-sim validation) strengthen our findings, trait proxies and diversification models have inherent limitations. Nonetheless, this study highlights sexual selection as a promising but underexplored driver of plant diversification, warranting future research integrating direct measures of sexual selection, ecological data, and genomic signatures of selection.



Gulliver's shipwreck: a case of miniaturisation in the genus *Asanada* (Chilopoda, Scolopendromorpha).

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POSTER SESSION 2 - **P02.309**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Body size is a key evolutionary trait and can influence rates of speciation and extinction, dispersal abilities, and geographic range sizes. In the centipede family Scolopendridae (27 mm to 105 mm), body size varies widely, yet the evolutionary dynamics of this trait remain understudied, particularly in smaller-bodied, morphologically cryptic genera. *Asanada* Meinert, 1884, is one of the smallest groups in terms of body size (25 mm to 30 mm) and distributed in old world tropics with high diversity in the Indian subcontinent. Unlike its other closely related members, the diversity of the group remains largely unexplored. The genus is monophyletic and it is nested within a much more cosmopolitan and large bodied *Scolopendra* Linnaeus, 1758 (120 mm), another specious group of the family Scolopendridae. It offers an interesting system to investigate the cryptic diversity and body size evolution. First, to propose a robust species hypothesis we used an integrative taxonomy framework and comprehensive sampling across peninsular India. We combined morphology, distribution and multilocus phylogenetics (28S, COI and 16S), and five species discovery methods (ASAP, GMYC, mPTP, PTP, bPTP), alongside one validation method Bayesian Phylogenetics and Phylogeography (BPP). The morphology based analyses suggested the presence of three distinct species. Morphological characters used to distinguish species in this group are largely

based on the differences in the ultimate leg morphology. The morphological diagnosis within *Asanada* is loosely reflected in the phylogenies that were reconstructed. We reconciled the results from species delimitation discovery methods to get a conservative estimate of distinct species and validated with BPP. The molecular phylogeny and species delimitation methods revealed ten independently evolving distinct putative species, far exceeding the three-species hypothesis proposed by morphology based analyses, highlighting substantial cryptic diversity.

We further investigated the evolution of body size using ancestral state reconstruction using a well-sampled comprehensive time-calibrated phylogeny of the family Scolopendridae. We compared the dynamics of body size evolution in the entire family with eight genera and seventy species. Trait evolution models (Brownian motion, ACDC exponential, and linear) consistently inferred medium ancestral body sizes with multiple independent shifts among extant taxa. Comparative analyses revealed that size diversification is more dynamic in the Scolopendrinae than in its sister subfamily Otostigminae. These findings highlight the instance of miniaturization, a relatively rare event among animals. The results validate the presence of cryptic diversity and elucidate the patterns and processes of body form evolution in these lesser-known centipedes.



Hybridization events among subterranean amphipods may be associated with the origin of nested adaptive radiations and morphological disparity

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POSTER SESSION 2 - **P02.288**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Adaptive radiations (AR) are bursts of massive speciation from a single ancestor associated with ecological divergence among descendants. One of the classical macroevolutionary questions is when and why AR unfold. A prerequisite for AR is ecological opportunity, a wealth of resources free of predators and competitors. A key question, however, is what maintains genetic diversity needed for prolonged periods of speciation and diversification. Many genome-wide studies of different AR suggest that a mechanism safeguarding genetic diversity through time is hybridization. We test this hypothesis using amphipod crustacean *Niphargus* living in groundwater of SE Dinaric Karst (W Balkans). Many *Niphargus* species tend to have extremely small ranges, which suggest limited dispersal and thereby low chances of hybridization, but also a stringent test to hybridization hypothesis. Materials and Methods: We studied SE Dinaric AR, an ecologically divergent clade of age ~10 My, within which is nested a younger AR of age ~3 My. We analysed 233 individuals belonging to 42 described or undescribed *Niphargus* species, of which 37 species (219 individuals) belonged to SE Dinaric AR, and 5 are other species (14 individuals) found in the region. The AR nested within the SE Dinaric AR counted 15 species (160 individuals). We tested for ancient and ongoing hybridization events, and evaluated ecological disparity of the ARs.

We used double digest restriction-site associated DNA sequencing (ddRADSeq) to generate SNP matrices for phylogenomic reconstruction and introgression analyses. After careful filtering of SNPs by minimising missing data while maximising phylogenetic resolution, we reconstructed the evolutionary history of *Niphargus* species. We then used admixture analyses and D statistics to infer recent and past introgression events across the adaptive radiation and outgroup species.

Next, we quantified functional morphological variation associated with habitat use and trophic ecology. The magnitude of functional diversity (corrected for phylogenetic independence and body size) of the target clades was visualized on PCA plots and quantified as a sum of cophenetic distance from the neighbour-joining dendrograms, and tested it for significance against 999 random assemblies of *Niphargus* species (N = 256 species).

Results: We detected traces of ancient and recent hybridization within and across species belonging to the AR. Specifically, there is evidence for an ancient hybridization of SE Dinaric AR ancestor with members of NE Dinaric clade and Greek clade. Furthermore, we found some evidence for hybridization among four clades within SE Dinaric AR. Finally, we detected hybridization among three the most intensively sampled species (*N. balcanicus*, *N. vjetrenicensis* and *N. hercegovinensis*), possibly yielding a phenotypically distinct hybrid species, *N. trullipes*. Overall, species within the adaptive radiation showed a higher amount of introgression than non-AR species. Disparity in habitat-related traits is higher than that of trophic ecology-related traits. Noteworthy, much of the functional diversity can be attributed mostly to diversity of the younger AR, nested within the SE Dinaric clade.

Conclusions: We found evidence for several hybridization events that likely took place prior to the onset of the SE Dinaric AR and nested AR. The results of our study are in line with results of studies using other model systems, suggesting that hybridization indeed presents a ubiquitous mechanism underlying AR. The evidence of hybridization could be particularly important for explaining general evolution in subterranean systems in which species' genetic diversity is generally lower.



Illuminating firefly diversity: molecular taxonomy and conservation of Italian Lampyridae

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POSTER SESSION 2 - **P02.272**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Firefly (Lampyridae) taxonomy remains critically unresolved on a global scale. The majority of described species are known only from adult males, and many have been defined based solely on limited morphological traits. As a result, species boundaries remain unresolved, with few studies applying integrative taxonomic approaches that incorporate molecular, morphological, and ecological data.

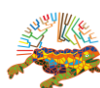
The Italian peninsula, recognized as a biodiversity hotspot, hosts 19 firefly species, 10 of which are endemic. Despite significant public interest and institutional concern for their conservation, fireflies in Italy remain underrepresented in key biodiversity databases such as GBIF and GenBank. This underrepresentation hampers research and conservation planning, which increasingly depend on robust, accessible data and accurate species-level identification.

Materials and methods: We collected a total of 171 firefly samples belonging to 13 species (Lamprohiza morio, N = 1; Lamprohiza splendidula, N = 1; Lampyris ambigena, N = 3; Lampyris bonvouloirii, N = 10; Lampyris fuscata, N = 15; Lampyris noctiluca, N = 7; Lampyris pallida, N = 2; Lampyris plurihomonyma, N = 2; Lampyris raymondi, N = 2; Lampyris sardiniae, N = 2; Lampyris somnambula, N = 3; Luciola italica, N = 4; Luciola pedemontana, N = 118). We extracted total genome DNA from firefly individuals collected throughout Italian mainland and islands, through standard procedures. DNA barcoding analyses were performed comparing obtained sequences with available public data of other lampyrid species.

Results: Phylogenetic tree reconstructions, allowed us to visualize relationships among taxa. Additionally, species delimitation tools (e.g., ABGD, GMYC, bPTP) supported the identification of cryptic species. For example Barcodes identified

Luciola pedemontana as a distinct, well-supported monophyletic clade and as the sister taxon of L. italica, with which it shares close morphological affinities. Furthermore, a low intraspecific genetic variation was found between L. lusitanica and L. pedemontana and between L. unmunana + L. papariensis. Genetic data obtained for the Luciola species can help to improve conservation measures for L. pedemontana, strongly required to protect this Italian endemic taxon, which is currently threatened by light pollution and environmental alterations. These results underscore the importance of molecular tools in resolving taxonomic uncertainty, especially for morphologically conservative groups such as Lampyridae.

Conclusions: To address broader knowledge gaps and improve access to firefly biodiversity data, the TETTRIs initiative (Transforming European Taxonomy through Training, Research, and Innovations) and the project L.U.C.E. integrates large-scale digitization of museum specimens across the Italian peninsula with the generation of new molecular, morphological, and geospatial data. Our approach prioritizes standardized, reproducible workflows that enhance taxonomic resolution and facilitate cross-institutional data sharing. Our work not only contributes to a more stable and reliable taxonomy of Italian fireflies but also supports conservation efforts at multiple levels. Accurate species delimitation is essential for assessing the conservation status of endemic taxa like L. pedemontana, which is increasingly threatened by habitat degradation, light pollution, and agricultural intensification. As insect populations decline worldwide, molecular taxonomy becomes a critical tool in documenting and protecting their diversity—ensuring that taxonomic uncertainty does not become a barrier to effective conservation.



Independent paths, shared landscapes: morphological and lineage diversification in Gymnophthalmidae (Reptilia: Squamata)

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POSTER SESSION 2 - **P02.296**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Gymnophthalmidae represents one of the largest and most diverse families of Neotropical squamate lizards, exhibiting remarkable ecological and morphological variation. This extensive diversity provides critical insights into the evolutionary processes that shape tropical biodiversity. To investigate these processes, we integrated genetic and morphological data to construct a phylogenetic framework and explore the macroevolutionary and biogeographic history of this clade.

We reconstructed phylogenetic relationships using genetic sequences obtained from GenBank, focusing on mitochondrial (12S, 16S, ND4) and nuclear (c-mos) markers. Phylogenetic inference was conducted using maximum-likelihood with IQ-TREE, and the tree was time-calibrated using secondary calibration points given the complete lack of gymnophthalmid fossils. Further, we employed Bayesian Analysis of Macroevolutionary Mixtures (BAMM) to estimate rates of speciation and morphological evolution, examining traits such as body size and limb development. Finally, ancestral ranges and biogeographic history were modeled using BioGeoBEARS, incorporating dispersal-extinction-cladogenesis, vicariance, and founder-event speciation models.

Our analyses recovered well-supported clades consistent with previous phylogenetic hypotheses and placed the diversification of Gymnophthalmidae between 78 and 82 million years ago. Significant lineage accumulation occurred primarily during the Miocene, with evolutionary rates varying substantially among clades. Notably, speciation and phenotypic evolution followed decoupled trajectories, suggesting independent dynamics of morphological change and lineage diversification.

Biogeographic reconstructions identified the Guiana Shield as the family's ancestral region, with dispersal emerging as the predominant biogeographic mechanism over vicariance. Major diversification hotspots included the Andes, Amazon, and Guiana Shield, with extensive species interchange during the Eocene and Oligocene. Contrary to previous hypotheses, our results indicate diversification from lowlands to higher elevations, rather than a strictly south-to-north progression in Andean lineages. These findings underscore the importance of dispersal events and ancient lineages in shaping the present-day diversity of Gymnophthalmidae across Neotropical ecosystems.



Integration Constrains Visual System Diversification in Ecologically Diverse Spiders

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POSTER SESSION 2 - P02.315, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Across many animals, ecologically diverse clades tend to exhibit high phenotypic and sensory system diversity. Visual systems often act as a crucial interface between animals and their environments, and hence their diversity might be expected to correlate with the diversity of ecological niches occupied by a group. Notably, in multi-eyed systems such as spiders possessing up to four pairs of camera-type eyes, the redundant arrangement of visual units enables different eye types to diverge under contrasting selective pressures. The marronoid clade (brown spiders) is a cryptic group of spiders that exhibits a diverse range of ecologies, and many occupy specialised habitats such as intertidal zones, tundra, deserts, caves, and a wide range of altitude gradients. Some species are free-hunting while others use webs for prey capture, and the group contains species that produce two different types of silk. However, whether the visual system of these spiders has evolved to match their diverse lifestyles remains largely unknown.

Materials and Methods: We used geometric morphometric methods on synchrotron radiation micro-computed tomography (SR μ -CT) scans of 45 species, representing all eleven marronoid families to study the size, orientation and arrangements of eyes across the phylogeny. We used Ornstein-Uhlenbeck models to test whether visual properties (eye size, arrangement, and orientations) evolve freely or in a constrained manner, and using simulations, we compared morphospace density between marronoids and 30 species from the species-rich RTA clade of spiders. Additionally, using covariance matrices, we also quantified the degree of modularity (i.e., the tendency of eyes to evolve as independent units) and integration (the tendency of all eye

pairs to evolve as a single unit) present in the spider visual system.

Results: We show that free-hunting species and those with sticky silk show increased evolutionary rates and morphological disparities in their visual systems as compared to web-building species and those without sticky silk, while the microhabitat occupied by the spider does not significantly correlate with visual system disparity. Our results also show that eye sizes, arrangements, and orientations evolve in a constrained manner, and this constraint is likely driven by a large amount of integration among the eye pairs i.e., the eye pairs evolve in a correlated fashion as one single unit, instead of evolving independently of each other. Moreover, our simulations reveal that marronoids have a significantly greater density of morphospace occupation and smaller morphospace branch lengths compared to 30 species of the species-rich RTA clade of spiders, despite both the clades occupying similar morphospace volumes.

Conclusions: Our analyses reveal how strong phenotypic integration (i.e., correlated evolution) among eyes can constrain the diversification of visual properties such as eye size, eye arrangement, and eye orientations, while certain ecological innovations such as loss of web-based hunting and production of sticky silk can still predict high evolutionary rates and increased morphological diversity. This balance between innovation and constraint highlights how complex trait coupling can shape macroevolutionary trajectories, even in lineages exposed to vastly different ecological pressures. Further work should investigate the developmental and genetic underpinnings of eye integration to understand how these constraints evolve and how they might be overcome during major adaptive shifts.



Landscape influences on gene flow in *Cochliomyia hominivorax* (Coquerel, 1858): implications for population connectivity in South America

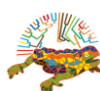
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POSTER SESSION 2 - **P02.269**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: The New World screwworm fly, *Cochliomyia hominivorax*, the etiological agent of myiasis, poses a critical challenge to animal health, particularly in regions with high livestock density. Its metapopulation dynamics, characterized by high gene flow among populations, complicate eradication efforts. Understanding how landscape configuration influences connectivity is essential for developing more effective management strategies. Landscape resistance, which represents how environmental features facilitate or restrict species movement, plays a fundamental role in explaining genetic variation among populations. Identifying high-resistance areas may contribute to population isolation, reducing genetic connectivity and supporting integrated management approaches. **Materials and Methods:** We investigated the influence of landscape on gene flow of *C. hominivorax* in South America, using 25 populations of this species. To quantify resistance, we used land-use maps and generated 10,000 resistance surfaces, maintaining the original landscape configuration but assigning random resistance values ranging from 0 (no resistance) to 100 (maximum resistance, representing a dispersal barrier) for different land-use classes. These surfaces were used to calculate resistance matrices between pairs of *C. hominivorax* populations using the ResistanceGA package (genetic algorithm for resistance surface optimization), optimizing the surfaces based on genetic variation observed between the populations. Model selection was performed using the Akaike Information Criterion (AIC), allowing identification of the resistance surfaces that best explained the genetic variation. **Results:** Genetic divergence, measured by F_{ST} , showed no significant correlation with geographic distance ($p > 0.05$), suggesting that other environmental factors influence connectivity. Among the 10,000 analyzed surfaces, only 13 exhibited significant correlations, with Pearson correlation coefficients ranging from $r = 0.338$ to $r = 0.512$. The most supported model (AIC difference < 2) indicated that pastures, grasslands, and mosaic landscapes containing mixed

vegetation had low resistance to gene flow. Conversely, forest areas and inland water bodies exhibited high resistance, while artificial areas (urban regions, roads) and mixed-use landscapes with forests showed intermediate resistance. Notably, regions with intensive agricultural activity also exhibited high resistance to gene flow, likely due to seasonal changes and pesticide applications. **Conclusions:** These findings underscore the critical role of landscape configuration in shaping the genetic connectivity of *C. hominivorax*. Population genetics is closely tied to landscape features, particularly due to species-specific dispersal patterns and biological traits. For *C. hominivorax*, we observed that 26% of the genetic divergence among populations is linked to the species' response to landscape composition. Incorporating landscape resistance data can support strategic decision-making by identifying key areas to restrict gene flow between populations. It is possible that landscape changes over time have played a role in modulating gene flow in *C. hominivorax*, influencing population connectivity and eventually contributing to genetic divergence processes. Exploring this relationship may help us understand how environmental dynamics influence genetic variation over time, offering new perspectives on the species' evolution. This insight can be crucial in designing effective control strategies, especially when combined with other management approaches, such as the sterile insect technique, biological control, and environmental management. When integrated, they can ultimately enhance the success of control programs for this parasitic disease. Additionally, environmental variables such as altitude, human population density, cattle density, and proximity to roads contribute to the landscape's resistance. These factors may impact the spread and survival of *C. hominivorax*, highlighting the need for landscape-specific management strategies that consider both ecological and anthropogenic factors to control the spread of this parasite.



Life-history trade-offs and lifetime reproductive success under high vs. low food availability in a spider

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POSTER SESSION 2 - **P02.285**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

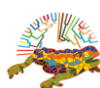
Background: Female lifetime reproductive success, defined as the total number of reproducing offspring, depends on both her genetics and environmental conditions. Food availability is a main environmental factor that may cause trade-offs between self-maintenance and reproductive success when food is limited. In species that use an income breeder strategy, a gravid female can invest the food energy into either own maintenance and survival or offspring number and/or quality. However, significant gaps remain in understanding how females adjust body maintenance and reproductive investment over their lifetime, particularly in response to food availability, in long-lived invertebrates with multiple reproductive events.

Materials and methods: We investigated this trade-off in the orb-weaving spider *Nephilingis cruentata*, a species in which females typically mate only once or twice due to paired genitalia, yet can live up to two years and produce more than fifteen egg sacs. We exposed females after mating to either low or high food (one or three flies twice a week, respectively) until natural death and also exposed the female offspring to the same food treatment as their mothers. We monitored maternal traits including body mass, the number and mass of laid egg sacs (ES), longevity, and two offspring traits. As

offspring traits, we monitored survival to adulthood and adult body mass.

Results: Low-food (LF) females lived longer and laid ES for longer time after mating, but their total ES number and the mass per ES was lower than that of high-food (HF) females. As a result, LF females reached only about one third the lifetime egg mass compared to HF females. In HF, average-age ES mass positively correlated with age-related changes in both ES mass and female body mass, whereas in LF female average-age ES mass negatively correlates with age-related changes in both traits. There was also difference between food treatments in a correlation between the average-age body mass and the change of ES mass over their lifetime. Specifically, average-age body mass correlated positively with age-related changes in ES mass in HF, but negatively in LF. In addition, we observed the food treatment effects on offspring traits: offspring of HF mothers had a higher likelihood of survival; their adult mass, however, was not significantly affected.

Conclusions: Overall, our findings align with life-history theory by demonstrating that limited resources drive strategic trade-offs between female body mass, longevity, reproductive investment, and offspring quality, with effects extending into the next generation.



Multilayer thin film produces recurrent iridescence in mammals

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POSTER SESSION 2 - P02.320, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Iridescent colouration is a vibrant structural colour that is widespread in nature, but in mammals is thought to be limited. Although multiple rodent and Eulipotyphlan species have been anecdotally described as iridescent, empirical evidence outside of the Chrysochloridae (golden mole) family is lacking. As iridescence in golden moles is created through a thin-film mechanism from a compressed cuticle structure, and given the structure of hair is highly conserved, we expect iridescence to be present, and produced by the same mechanism, in mammals that share similar hair properties. Here, we test this hypothesis by first collecting and analysing existing

documentation of iridescence in mammals, finding written evidence spanning 25 genera across eight mammalian families. We then identified the underlying mechanisms of iridescence for 14 species from Rodentia and Afrosoricida (including one outside Chrysochloridae), and showed that iridescence in these species is created through the same system of thin, alternating layers of keratin and likely lipid rich material within the cuticle, in similar proportions as Chrysochloridae. These data suggest that iridescence in mammals is more common than thought, adding another dimension to mammal colouration research, and demands further evolutionary exploration.



Non-random loss of ecological roles following extinction and extirpation on island bird communities

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¹CREAF, ²GEES

POSTER SESSION 2 - **P02.290**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Anthropogenic pressures have driven the extinction of thousands of species worldwide. These extinctions frequently cause cascading effects on ecosystems through the loss of their often-unique ecological roles. Despite growing awareness of these impacts, our understanding of the generality and spread of these functional losses remains limited. This is mostly due to i) the lack of ecological trait data - such as dietary niche - for extinct species, and the fact that ii) local extirpations, especially on islands, are often overlooked in broad-scale analyses despite their ecological consequences for local biological communities.

In this study, we address both gaps by first compiling a new global database of island-level local extirpations, gathering information from published literature. This database includes data on 1,905 local extirpations, including 785 bird species extirpated across 397 islands. To obtain data on the ecological roles of extinct species, we used a machine learning Random Forest algorithm that inferred missing information using morphological, ecological, and phylogenetic traits from all extant bird species worldwide. Our machine learning models reliably estimate ecological roles, with an accuracy of approximately 85% when tested on extant species. Using this method,

we inferred the diets of extinct species and examined if certain ecological are more vulnerable to loss than others while examining the geographical factors influencing this vulnerability. Our findings show that the loss of ecological roles associated with bird extirpations on islands is non-random; certain roles are disproportionately affected by human threats and the attributes of islands. Additionally, we found that island communities vary in their vulnerability to functional loss, with some ecological roles being more frequently lost than others.

This work helps shed light to our understanding of the functional consequences of both local and global extinctions, linking long-term impacts of anthropogenic pressures with ecological patterns of extinction. We underscore the importance of accounting for local extirpations when assessing functional diversity loss and highlight the need for more comprehensive databases that include both global and local extinction events. Our findings could inform future conservation efforts by identifying both species and/or ecological roles most at risk as well as by providing a deeper understanding of the dynamics shaping present-day avian community composition.



Phylogenetic inference from an incomplete fossil record

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POSTER SESSION 2 - P02.312, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Morphological and temporal data from fossils has become an increasingly important part of phylogenetic inference. It is a crucial part of the construction of time trees, which open the opportunity to test a wide range of evolutionary hypotheses via comparative methods.

However, the fossil record is known to be incomplete: Organisms might not fossilize, and fossils might be destroyed by erosive and taphonomic processes. However, gaps in the fossil record are not random, but can be predictably linked to external drivers of stratigraphic architectures such as changes in sea level.

We explore effect of the incompleteness of the fossil record on the precision and accuracy of phylogenetic inference. For this, we combine forward models of the rock record with simulations of the fossilized birth-death model to generate a synthetic fossil record. We explore how well the true evolutionary history (tree topology and model parameters) can be recovered from stratigraphic architectures controlled by varying external drivers. Our results will elucidate how data sparsity introduced by geological effects influence tree inference and identify scenarios that are best suited for the recovery of evolutionary change from fossil data.



Phylogenetic Oscillating Ornstein-Uhlenbeck process: analysis and application.

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*POSTER SESSION 2 - **P02.311**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM*

We characterize theoretical properties of the 2-dimensional Ornstein-Uhlenbeck process which results from a complex-eigenvalued adaptation rate matrix, and how it fits in a phylogeny as a macroevolutionary model. Bayesian settings are utilized, and a prior distribution is proposed that can induce a

certain evolutionary behavior into the resulting posterior model. As a proof of concept, we implement our method on the European butterflies data, where we test hypotheses regarding coevolution of somatic and sensory traits.



Phylogeography and Emergence of the Southernmost Orthohantavirus Lineage: A Novel Clade in the Magellanic Rainforests of Chile

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POSTER SESSION 2 - **P02.274**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Hantaviruses (genus Orthohantavirus) are zoonotic pathogens transmitted primarily by rodents, causing Hantavirus Cardiopulmonary Syndrome (HCPS) in the Americas. Since its initial identification in the United States (1993) and Chile (1995), Orthohantavirus andesense (ANDV) has been the primary causative agent of HCPS in southern South America, with the long-tailed pygmy rice rat (*Oligoryzomys longicaudatus*) as its main reservoir in Chile and Argentina. The ANDV genome comprises three RNA segments (Large, Medium, and Small), with phylogenetic studies revealing three major clades corresponding to distinct ecogeographic regions in Chile: Mediterranean, Valdivian Rainforests, and Patagonian Rainforests. However, the potential presence of ANDV in the southernmost Magellanic Rainforests—an extension of the reservoir's range—remained unexplored.

We hypothesized that ANDV circulates in *O. longicaudatus* populations within the Magallanes Region (Chile) and forms a distinct lineage phylogenetically related to—but divergent from—the Patagonian clade. To test this hypothesis, we conducted rodent trapping across three Magellanic Rainforest sites, followed by serological screening (SIA) and genomic sequencing (Sanger) of ANDV-positive samples. Among 61 captured rodents, three were seropositive, and we

successfully sequenced partial Small (S) and Medium (M) segments, revealing a novel ANDV lineage—the southernmost reported globally.

Phylogenetic analyses (Maximum Likelihood/Bayesian inference) confirmed that ANDV in Chile segregates into four well-supported clades, each associated with a major ecoregion: Mediterranean, Valdivian rainforests, Patagonian rainforests, and now, Magellanic rainforests. Genetic divergence estimates suggest prolonged isolation of the Magellanic lineage, likely driven by biogeographic barriers and host population structure. Notably, the newly identified clade shares a common ancestor with Patagonian strains but exhibits unique substitutions in the glycoprotein (Gn/Gc) and nucleocapsid (N) coding regions, potentially influencing viral fitness and host adaptation.

These findings extend the known distribution of ANDV and underscore how ecoregion-driven evolution shapes hantavirus diversification. The identification of this southernmost lineage emphasizes the need for enhanced surveillance in understudied regions, as well as a deeper understanding of hantavirus evolution and distribution. Such knowledge may be critical for developing effective public health strategies to mitigate future outbreaks. ANID - FONDECYT 1230881



Population genomic structure and geographical differentiation of a spiny crawler mayfly, *Drunella ishiyamana*, in erosive and flashy streams in Mongolia

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POSTER SESSION 2 - **P02.282**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Understanding fine-scale genetic differentiation is crucial for revealing evolutionary processes in aquatic insects, shaped by habitat environmental conditions. Among them, mayflies play a vital ecological role as primary consumers in freshwater ecosystems, functioning as herbivores and detritivores while contributing to nutrient cycling. Their sensitivity to temperature fluctuations and hydrological changes makes them excellent bioindicators of water quality and ecosystem health, particularly in the context of climate change. In Mongolia, streams are notably erosive and flashy, characterized by high variability in water flow velocity and high sediment transport due to seasonal precipitation and sparse riparian vegetation. Within these dynamic environments, the spiny crawler mayfly *Drunella ishiyamana* serves as a promising model for studying population structure and local adaptation in response to the challenges posed by such unstable freshwater systems. This study explores the population genomics of *Drunella ishiyamana* across seven sites in Mongolian streams to investigate patterns of population structure.

Materials and methods: A total of 66 individuals from the seven populations of *D. ishiyamana* were analyzed using Genotyping-by-Sequencing (GBS) with the Stacks de novo pipeline. After filtering for loci with no missing data and a minor allele frequency ≥ 0.05 , 17,592 SNP loci were retained. Genetic diversity metrics, pairwise F_{ST} , and STRUCTURE analysis ($K = 4$) were conducted using the Stacks populations module and STRUCTURE software.

Results: All populations exhibited high levels of polymorphism ($P > 0.76$) and nucleotide diversity (π) ranged from 0.301 to 0.330. Genetic diversity analysis showed moderate variation, with heterozygosity ranging from 0.298 to 0.371 and inbreeding coefficients between -0.097 and 0.055. One geographically remote population consistently showed higher genetic diversity and distinctiveness, with moderate F_{ST} values of 0.09 compared to other populations. The distinct genetic profile of DI-T2, located in Gorkhi-Terelj National Park, suggests that it may represent a unique evolutionary lineage within the region's stream ecosystems. STRUCTURE analysis revealed four genetic clusters. Three populations—DI-05, DI-06, and DI-08—from the Khangai Mountains, located in central Mongolia, exhibited substantial admixture. In contrast, the DI-T2 population consistently showed moderately high levels of genetic differentiation ($F_{ST} > 0.09$), reinforcing its potential role as an isolated lineage. Meanwhile, DI-05, DI-06, and DI-08 were the most genetically similar groups, supporting the possibility of recent gene flow or shared ancestry.

Conclusions: This study investigates the population structure of *Drunella ishiyamana* across seven sites in Mongolian streams to uncover patterns of population genomics. These streams, which traverse the vast steppe landscape, are highly susceptible to erosion and drought. The results reveal clear population structure and localized genetic differentiation in *D. ishiyamana*, likely shaped by the dynamic and rapidly changing conditions of these aquatic systems.



Repetitive element density in micro and macro avian chromosomes is shaped by the interplay of 3D architecture constraints and recombination rate

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POSTER SESSION 2 - **P02.305**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Transposable element density can drastically vary between chromosomes, and it is usually shaped by population genetics parameters like recombination rate. However, the repeat density between macro (> 40Mb) and micro (< 20Mb) chromosomes of bird species can be very similar despite very different rates of recombination. Recombination rate is higher in microchromosomes which predicts a lower repetitive element content with respect to macrochromosomes, but we do not observe this pattern. Here, I hypothesize that the peculiar 3D architecture and nuclear location of microchromosomes hinder the purging of transposable elements by making

microchromosomes more vulnerable to be target of transposable element insertions. High rates of recombination counteract the accumulation of transposable elements, however, the efficacy of selection is not enough to contrast the higher mutational input and to keep microchromosomes deprived of transposable elements.

I test the hypothesis that microchromosomes are preferential targets of transposable element insertions because of their 3D architecture by leveraging a vast and phased SNP and long read-based structural variant dataset from 200 wheatears (*Oenanthe* genus) together with HiC and Iso-seq transcriptomic data.



Scaling the Tree: How Clade Age and Size Govern Species Richness Gradients

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POSTER SESSION 2 - P02.299, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Species richness gradients are often viewed as universal. However, their variation has rarely been systematically evaluated, especially across the phylogeny. It is possible that well-known gradients, such as the latitudinal diversity gradient, hold consistently only for clades of certain age and size. Here, we analyze how species richness changes with latitude, environmental productivity and temperature for all clades within birds and mammals (~15,000 species). We use multiple gradient measures (raw richness, log-transformation, log-log transformation, parametric and non-parametric correlations), which we correlate with clade age and size to identify phylogenetic trends. To tease apart biological and statistical causes of the trends, we construct null models and use simulations. We find that, as clade age and

size increase, the gradients become steeper and more consistent. Yet, even relatively large clades (up to 500 species) regularly show reverse gradients (35% of the clades within birds and mammals). Similar, albeit less pronounced, phylogenetic trends arise even within simulations and null models, suggesting that the trends might be partly statistical. We conclude that species richness gradients follow phylogenetic trends, which arise equally for biological and statistical reasons. Based on our results, we formulate guidelines on how to measure the gradients for more meaningful cross-taxa and cross-study comparisons, depending on the tested theory and research purpose. Knowledge of the phylogenetic trends paves the way toward a synthesis of the wide variety of gradients reported across taxa and previous studies.



Sex roles, breeding systems and sexual dimorphism: combining studies of microevolution and macroevolution

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POSTER SESSION 2 - P02.271, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Sex roles, breeding systems and traits associated with sexual selection exhibit immense diversity across the animal tree of life. Males and females often adopt different strategies over reproduction and the causes of these strategies have stimulated vast theoretical empirical advances in evolutionary biology that have implications for sexual dimorphism, life history evolution, population ecology, speciation and biodiversity loss. Our research group is using shorebirds (sandpipers, plovers and allies) as model organisms to investigate microevolutionary processes of breeding systems in multiple study sites on several continents. Parallel with these studies, we are spearheading phylogenetic comparative analyses of mating systems, parental care and sexual size dimorphism. The combination of micro- and macro-

evolutionary investigations provided new insights into evolutionary processes beyond breeding systems. First, they proposed a novel outcome of sexual selection for speciation. Second, they highlighted the roles of an underappreciated population property – the social environment as represented by the adult sex ratio – for sexually selected traits. Third, they uncovered a non-conventional perspective on sex role evolution by challenging the role of anisogamy driving the Darwin-Bateman paradigm. In this presentation, we overview the major conclusions of 35 years of field investigations and phylogenetic analyses. In addition, we aim to identify the key components, assumptions and implications of these investigations, and propose future studies to break through the barriers of our current knowledge.



Task Goals Shape Cultural Attractors: An Experimental Study of 3D Artifact Evolution

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POSTER SESSION 2 - **P02.316**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Cultural evolution often involves the convergence of artifacts or behaviors towards stable forms, known as cultural attractors. Cultural Attractor Theory suggests these attractors arise from systematic transformations during transmission, guided by cognitive biases or environmental constraints. While foundational, empirical understanding of how different types of goals - representing distinct cognitive biases or selective pressures - specifically shape the properties and dynamics of cultural attractors, particularly in continuous multi-dimensional trait spaces, requires further investigation. Clarifying this link is essential for understanding how goal-directed behavior drives cultural adaptation and the emergence of stable technological or aesthetic forms.

Materials and methods: We employed a transmission chain experiment involving iterative 3D artifact design in a 5-dimensional parameter space. Each isolated transmission chain included three generations. Participants were recruited from three distinct populations chosen to span cultural and socioeconomic diversity: Prague, Czech Republic (WEIRD university students); Buea, Cameroon (non-WEIRD university students); and Bokwango, Cameroon (non-WEIRD rural village). Participants were instructed to use a custom 3D object configurator to design an artifact optimized for a given task. Within each population, participants engaged in either an Engineering task (minimize rolling time of a 3D printed version down a sloped ramp), an Aesthetics task (maximize peer-rated visual appeal of a 3D printed version), or an Averaging task (estimating the mean of parent artifacts, serving as a baseline). Participants of the first generation were not shown any artifacts, however, participants in the subsequent generations were provided two or three artifacts from the previous generation for inspiration. We tracked the artifact parameters, 3D printed the artifact, and measured its performance metrics (rolling time, aesthetic ratings) for artifacts across three generations within each chain.

Bayesian hierarchical models are used to compare the dynamics of convergence, the location, and the stability (variance) of attractors across the three task conditions.

Results: The analyses focus on characterizing and comparing the attractor landscapes generated under different goal conditions within the 5D parameter space. Our experience shows that distinct artifact morphotypes arise and compete during the simulated evolution, not necessarily based on their fitness. We expect to identify distinct cultural attractors corresponding to each task. Preliminary results suggest that the Engineering task, with its objective physical constraints, leads to faster convergence towards a more sharply defined attractor aligned with functional optima (low rolling time). The Aesthetics task, relying on subjective judgments, exhibits slower convergence, potentially towards broader or multiple attractor regions corresponding to different aesthetic optima, possibly showing more influence from initial conditions or population-specific preferences. The Averaging task serves to quantify baseline convergence due to transmission noise and statistical effects. The results, which will be presented in full at the conference, comparing the trends across tasks via Bayesian models will quantify the evidence for differences in attractor location, sharpness, and the speed at which they are reached.

Conclusions: This study experimentally demonstrates how specific task goals provoke distinct factors of attraction and shape the process and outcomes of cultural evolution within a continuous trait space. The findings demonstrate that the nature of the task fundamentally influences the dynamics and outcomes of cultural transmission in a continuous trait space. This clarifies the mechanisms underlying goal-directed cultural adaptation and the emergence of stable cultural forms, contributing to our understanding of innovation, problem-solving, and the evolution of technology and design.



The Evolutionary Dynamics of Transposable Elements in the Bengal Slow Loris (*Nycticebus bengalensis*)

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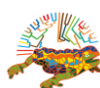
POSTER SESSION 2 - **P02.297**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Endogenous retroviruses (ERVs) are a class of transposable element (TE) and are the result of an exogenous infections of retroviruses in germline cells resulting in integration within the host genome. ERVs constitute between 8 and 10% of genomes, contributing to genome size, viral resistance and traits. The majority of ERV research has focused on humans and other great apes, and their impact on genome evolution and involvement in disease has been well established, but little is known if these observations hold across the wider primate order. Here we investigate the impact of ERVs in the smaller primates (Strepsirrhines) with a particular focus on the endangered slow loris (*Nycticebus*). Our previous work identified a novel ERV (LERV1) in the publicly available *N. bengalensis* and *N. coucang* reference genomes. Investigation of the genomic distribution of LERV1 indicated that the two reference genomes belong to the same species (*N. bengalensis*), due to the high similarity of the majority of insertions (this conclusion was reinforced by analysis of the mitochondrial genes). Here we present a more complete analysis of the ERV and TE content in the *N. bengalensis* genome; in addition to the two aforementioned publicly available reference

genomes, we sequenced an additional *N. bengalensis* individual which allows us to conduct population level genetic analysis of ERVs in this species.

Materials and methods: We used RepeatModeler2 for initial identification of novel repetitive families of TEs within the *N. bengalensis* genomes. Custom bioinformatic pipelines were used to conduct further analyses on the genomic distribution and population dynamics of the identified TE families.

Results and Conclusions: Initial searches of the slow loris genome with known repetitive elements revealed much lower levels of ERVs compared to great apes. By searching for novel elements, we found a large amount of novel ERV families within the *N. bengalensis* genomes spanning multiple retrovirus genera and having varying ages of insertion. Previous searches of repetitive elements within other strepsirrhine primates (such as lemurs) reported much lower levels of elements such as ALUs – however this relied on a homology based approach to known ALU sequences. Our analysis provides a much more comprehensive insight into the impact of TEs on the genome evolution of strepsirrhines.



The role of temperature and life cycle variation in Caudata diversification

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POSTER SESSION 2 - **P02.286**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Species richness varies widely among taxonomic groups, and understanding the mechanisms driving these differences remains a challenging question. Recent developments in macroevolutionary methods based on phylogenetic trees have highlighted the role of variation in diversification rates—the balance between speciation and extinction—in shaping these patterns. However, the factors that influence macroevolutionary dynamics are still poorly understood. Here, we investigate the role of both abiotic and biotic factors in shaping diversification rates in Caudata, an amphibian clade that exhibits remarkable diversity in species, phenotypes, ecologies and life cycles, as well as striking disparities in species richness across its lineages.

Materials and methods: We used the most recent and comprehensive time-calibrated phylogeny of the clade, which includes 763 species of salamanders and newts. We assessed the relative contributions of clade age and macroevolutionary rates to species richness using two branch-specific diversification models. We further tested the influence of past temperature, continental fragmentation, and life cycle in Caudata diversification using a suite of diversification models, such as environment-dependent birth-death and state-dependent speciation and extinction models.

Results: Our results indicate that diversification rate, rather than clade age, is the primary explanation for differences in species richness within Caudata. Notably,

high speciation rates are detected in the most recent lineages of Ambystomatidae, in Tylotriton-Echinotriton, and in Desmognathus, as well as in early-diverging branches of the three most species-rich families (Plethodontidae, Salamandridae, and Hynobiidae) highlighting early radiations followed by slowdowns in diversification. Past temperature fluctuations show a positive correlation with speciation rates in Caudata, with diversification peaking twice during the evolutionary history of the group: once during the Cretaceous Thermal Maximum (~95–85 Ma) and again during the Early Eocene Climatic Optimum (~54–49 Ma). However, the weak evidence for temperature-dependent diversification in Plethodontidae, Salamandridae, and Hynobiidae suggests that these clades may have followed clade-specific evolutionary trajectories. Unexpectedly, variation in life cycle does not appear to significantly influence diversification rates. Instead, other traits—unidentified in this study—likely drive speciation and extinction patterns, underscoring the complexity of macroevolutionary processes in Caudata.

Conclusion: Our findings show the role of diversification rate variation in shaping salamander species richness and suggest that abiotic drivers, such as temperature, contribute to these patterns. However, the influence of additional ecological and evolutionary factors remains to be explored to better understand the mechanisms underlying the diversification of Caudata.



The speciation continuum in bloom: Incomplete lineage sorting, gene flow, and reticulate evolution in rapidly diverging plant lineages

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POSTER SESSION 2 - P02.319, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: The remarkable diversity of life on Earth stems from evolutionary processes operating across different spatial and temporal scales. Among these, species diversification occurs through various mechanisms and at widely varying rates. Understanding the factors driving these rate shifts, especially the conditions that trigger bursts of diversification over short timescales, remains a central challenge in evolutionary biology. This difficulty is heightened in cases of recent and rapid divergence, where incomplete lineage sorting (ILS), hybridization, and ongoing gene flow obscure evolutionary relationships and complicate species delimitation.

Materials and methods: In this study, we investigated the evolutionary history and species boundaries within a group of recently diverged *Petunia* lineages shaped by pervasive gene flow. We integrated phylogenomic, population genetic, and species delimitation approaches to reconstruct lineage relationships and assess whether these lineages represent distinct species or points along a speciation continuum.

Results: By applying methods capable of accounting for both ILS and gene flow, we revealed that most lineages are not fully independent evolutionary units but rather occupy intermediate positions along this continuum. Gene flow played a key role during diversification, blurring species boundaries and generating reticulate evolutionary patterns.

Conclusions: Our findings demonstrate that traditional phylogenetic trees may oversimplify relationships in such systems, while phylogenetic networks offer a more accurate representation of evolutionary history. Comprehensive, integrative analyses like those employed here are essential for capturing these complex dynamics. Ultimately, only four lineages could be confidently recognized as distinct species, while the remaining represent ongoing, unresolved divergence. These results underscore the need to refine species delimitation frameworks for systems characterized by recent divergence and extensive reticulation.



Transposable Element Diversity in The Mountain Chicken Frog (*Leptodactylus fallax*)

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POSTER SESSION 2 - P02.304, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Transposable elements can have a considerable impact on host genome evolution. Here we focus on the class of TEs derived from viruses, endogenous retroviruses (ERVs), which comprise a significant proportion (~10%) of vertebrate genomes and have provided a useful source coding and regulatory sequences throughout vertebrate evolution. Our previous work on the reference genomes of Anurans has highlighted an abundance of novel ERV families across the order, which prior to this had been underrepresented in ERV research. Here we focus on the critically endangered mountain chicken frog (*Leptodactylus fallax*), found on the islands of Dominica and Montserrat, to provide insight into ERV population dynamics across a species genome. We sequenced and assembled the genomes of two individuals (one from each population) and screened these individuals for ERVs.

Materials and Methods: To screen the mountain chicken frog genomes for ERVs we used RepeatModeler2 for initial identification of novel ERV families. We then used custom bioinformatic pipelines and manual curation for subsequent analysis of RepeatModeler2 results, including

phylogenetic comparison of all retroviral genes for comprehensive classification of insertions.

Results and Conclusions: We identified multiple different families of ERVs in *L. fallax*, predominantly belonging to the epsilonretroviral genus, similar to our previous findings in anurans. Several of these ERV families are novel to *L. fallax* and have not previously been described in any vertebrate genome. Building on our earlier study of ERVs in the anuran order, we have further defined ERV classification, specifically regarding epsilonretroviruses. We have identified anuran ERV families with the potential for recent activity, retaining intact genes with little divergence between their long terminal repeats. By comparing ERV diversity within two geographically separated populations of *L. fallax*, we explore how population-level processes, such as genetic bottlenecks, shape genomic landscapes regarding ERVs. These findings provide a microevolutionary perspective on ERVs in the host genome which contribute to our understanding of macroevolutionary patterns of viral endogenization and their potential impact on vertebrate genome evolution.



Using contact zones to inform species delimitation in the gray zone of speciation: delimitation of species within the *Acanthodactylus erythrurus* complex

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POSTER SESSION 2 - **P02.298**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

One of the main goals of phylogeography is to identify the mechanisms that generated the current spatial distribution of genetic diversity within and between species. Among these mechanisms, detecting the action of vicariance and speciation requires to detect phylogeographic breaks and intrinsic barriers to gene flow that ultimately leads to speciation. In this work we used the red-tailed spiny-footed lizard (*Acanthodactylus erythrurus*) as a model to explore the evolutionary drivers of diversification in this complex. *Acanthodactylus erythrurus* is a widespread “species” of the Lacertidae family, ranging from the Iberian Peninsula to North Africa. It is a species that harbours a high intraspecific diversity both genetically and morphologically, with at least 11 mitochondrial lineages recognized. This remarkable variability encompasses spatial discontinuities that results in geographically structured and genetically distinct pools of individuals. However, distinguishing population-level variation from species-level divergence has often remained a challenge, especially in the early stages of speciation when introgression and admixture are only partially restricted—an situation referred to as the gray zone of speciation. A previous study on *A. erythrurus* relying on a small number of nuclear markers have faced such limitations to effectively determine the appropriate taxonomic level of the lineages within the species complex. In this study we aimed to detangle this intricate genetic variation using a genome-wide approach to describe the phylogenetic relationships and demographic history of the species, ultimately aiming at identifying the contributions of vicariance and speciation to the diversification of this group.

We used RAD sequencing method to obtain a genome-wide SNPs dataset of 548 specimens, covering most of the range of the species. We produced a Likelihood tree using the program iqTree to compute phylogenetic structure and delimit clusters. To assess potential admixture

between populations and/or hybrid lineages we performed a Principal Component Analysis (PCA) and used the Bayesian clustering algorithm STRUCTURE to investigate population structure. We then generated a species-tree using the package SNAPP from the program BEAST to infer phylogenetic relationships and account for possible incomplete lineage sorting (ILS) also checking for topological conflicts that might indicate introgressions. Finally, we analysed the degree of reproductive isolation in the contact zones through geographic clines between all pairs of parapatric populations.

The phylogenetic tree recovered the 12 clades previously described within the complex, of which 8 were explored in the population structure analysis. Although the geographical clines suggested that populations are reproductively isolated, with steep transitions of genetic frequencies between populations, our analysis of population structure supports a reticulated evolutionary history in the *A. erythrurus* complex. Our results suggest a diversification with old introgressions and the formation of at least one hybrid lineage.

Our study highlights the complexity of the speciation process within the *A. erythrurus* complex, where historical introgression and hybridization have played a significant role in shaping current genetic patterns. The analysis of reproductive isolation across contact zones enabled a more accurate assessment of gene flow between populations, allowing us to better determine the appropriate taxonomic status of the lineages within the species complex. By integrating genome-wide data with multiple analytical approaches, we provide a more nuanced understanding of lineage diversification within the gray zone of speciation, offering clearer criteria for species delimitation and contributing to the reduction of biodiversity knowledge shortfalls.



Using Morphological Traits for the Species Delimitation under a Polymorphism- Aware Phylogenetic Models approach

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POSTER SESSION 2 - P02.302, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

In evolutionary biology, genomic sequences and morphological data play a crucial role in the study of population histories. However, for species tree inference and species delimitation these kinds of data have been treated as different datasets for analytical purposes, weighting one over the other or just analysing them sequentially without a true relationship, which in some scenarios could lead to contradictory results. I intend with this project to develop a new methodology for species delimitation that combines both continuous morphological traits using Brownian motion (BM) and Ornstein-Uhlenbeck (OU) processes and multi-locus genomic data, the last using the advantages the Polymorphism-aware Phylogenetic Models (PoMos) framework offers. Combining these two frameworks into a common model that explicitly considers both types of data it will be useful for

overcoming common phylogenetic issues like Incomplete Lineage Sorting. For the implementation I am using the open-source probabilistic language RevBayes, which offers several advantages for phylogenetic modelling and validation. To test the model, multiple datasets will be generated using the traits and genes simulator implemented as part of the iBPP software.

Furthermore, I am going to apply the new approach to real morphological data and nuclear sequences from museum samples of South American Didelphidae and Thomasomyini species, which are known to have complex evolutionary histories, and compare with other species delimitation algorithms; to improve the current knowledge we have of the evolutionary history of this taxon.



Why do kangaroos hop? Inferring ancestral foraging and locomotion traits to trace the evolution of hopping among marsupials

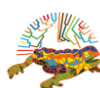
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POSTER SESSION 2 - **P02.275**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

There is a close relationship between the locomotion of animals and their occupation of foraging ecospace. Mammals display a wide range of locomotory modes, among which bipedal hopping is thought to have evolved independently multiple times within both marsupials and placentals. However, marsupial kangaroos, wallabies, bettongs and potoroos (Macropodoidea, Diprotodontia), and placental springhares (Pedetes, Rodentia), are the only extant hopping mammals above 500g. In this study we consider how evolutionary contingencies associated with foraging and locomotion might have driven these phylogenetically distant taxa to converge on a similar hopping gait mode; essentially asking why do

kangaroos hop? Here, phylogenetic ancestral trait reconstructions of Australasian macropods and African springhares were performed to trace the evolution of foraging height and locomotion. The results highlight that the ancestry of medium to large-bodied ($\geq 500\text{g}$) hopping macropods was likely subject to selection pressures for efficient terrestrial locomotion at faster gaits, as well as digging and climbing; a condition that could have caused evolutionary conflicts, especially at larger size. Evolving bipedal hopping could have resolved such functional conflicts by uncoupling hindlimb specialisation (terrestrial locomotion) from forelimb specialisation (digging/climbing).



A computational model for the macroevolutionary bias towards simple leaf shape in flowering plants.

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MEETING ROOM 114, August 20, 2025, 10:30 AM - 12:30 PM

Using large-scale phylogenetic trees coupled with herbarium data, we demonstrate that transitions from complex to simple leaf shapes have occurred at significantly higher rates than the reverse throughout the evolutionary history of angiosperms [1]. We then compare this macroevolutionary pattern to transition rates derived by applying random mutations to a well-established computational model of leaf development [2]. Our results show that mutation alone is sufficient to reproduce the observed evolutionary bias towards simple leaf forms. This suggests that developmental bias may have played a key role in shaping the evolutionary trajectory of leaf morphology. These findings are consistent with recent evidence that a developmental

bias towards phenotypic simplicity similarly influences the evolution of molecular structures such as RNA secondary structures and protein quaternary assemblies [3].

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2. Runions, A., Tsiantis, M. & Prusinkiewicz, P. A common developmental program can produce diverse leaf shapes. *New Phytologist* 216, 401–418 (2017).
3. Johnston, I. G. et al. Symmetry and simplicity spontaneously emerge from the algorithmic nature of evolution. *Proc. Natl. Acad. Sci. U.S.A.* 119, e2113883119 (2022).



A general evolutionary model for the emergence of novel characters from serial homologs

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MEETING ROOM 114, August 19, 2025, 2:00 PM - 5:00 PM

The origin of morphological characters with novel forms and functions is among the most fascinating phenomena in evolution, and understanding its underlying principles has been a fundamental goal of evolutionary biology. An essential means by which novelties evolve is the divergence of repeated body parts, known as serial homologs, into various forms, as in the case of tetrapod limbs and insect wings. However, the mechanisms underlying such processes are poorly understood, with systematic investigations hindered by the lack of a generalized model that links selective, genetic, and developmental mechanisms and the expected patterns of evolutionary dynamics. To fill this gap, we propose a generalizable model for the evolution of serial homologs that is founded on recent advances in developmental biology, where the development of each serial homolog is

controlled via a hierarchical gene regulatory network consisting of genes that specify character identity and those that encode the specific phenotypic state. Under this model, we investigated two types of evolutionary novelties: divergence in character states between body parts with conserved identities and switching of character identity by turning on and off master regulators. Using population genetic simulations, we demonstrate how selection and developmental constraints interact to shape dynamics of phenotypic evolution and conditions under which each type of novelty is likely to evolve. Together, our results provide general insights into how novelties could evolve from serial homologs, and offer a modeling framework where the developmental evolution of a broad range of phenotypic characters can be studied.



Applications of stochastic reproduction-selection models for the study of non-equilibrium dynamics across different evolutionary time-scales

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MEETING ROOM 114, August 19, 2025, 2:00 PM - 5:00 PM

Models in molecular evolution are typically separated into two groups, population genetic or microevolutionary models that describe intra-species genetic variation and phylogenetic or macroevolutionary models that describe inter-species genetic variation. Common to both settings is that derivation of analytical results and methodological developments frequently rely on the stationarity assumption, which corresponds to a stationary allele frequency distribution at the microevolutionary scale and a stationary sequence content at the macroevolutionary scale. Yet, most natural populations are not in equilibrium, since many evolutionary processes fluctuate over time and give rise to non-equilibrium conditions. To study the resulting non-equilibrium dynamics, we formulate a stochastic reproduction-selection model,

which in the scaling limit converges to a Poisson random field model. This allows us to obtain exact analytical expressions of relevant nonstationary allele frequency spectra, and to formulate micro- and macroevolutionary measures of key evolutionary processes: We delineate the influence of fluctuations in population size on the selection-drift balance predicted under the nearly neutral theory. We investigate if a combination of micro- and macro-evolutionary signatures of GC-biased gene conversion carry information about the dynamic of the recombination landscape. Moreover, we anchor the Poisson random field model within a mutation-selection framework, which enables us to study the effect of shifts in the fitness landscape on molecular signatures of selection.



Bridging micro and macroevolution: Insights from chromosomal dynamics in holocentric true sedges

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MEETING ROOM 114, August 20, 2025, 10:30 AM - 12:30 PM

Chromosomal speciation theory primarily focuses on monocentric chromosomes, where a single centromere governs segregation. However, holocentric chromosomes, characterized by dispersed kinetochores (holocentromeres) along the chromosomes, challenge this paradigm. True sedges (Carex, Cyperaceae), with their holocentric chromosomes and remarkable karyotypic diversity ($2n = 10\text{--}132$), offer a unique system to explore chromosomal evolution. Unlike monocentric species, where fissions often lead to acentric fragment loss and fusions to segregation errors, holocentric chromosomes in Carex allow for the preservation of fragmented segments due to distributed kinetochore function. This facilitates extensive chromosomal rearrangements (CRs) as a primary driver of their diversification.

Carex exhibits striking chromosome-number polymorphism, even within populations (e.g., *C. scoparia* $2n=56\text{--}70$, *C. laevigata* $2n=69\text{--}84$, *C. helodes* $2n=68\text{--}75$). Experimental crosses reveal that reproductive isolation intensifies with CR accumulation, resulting in hybrid seed germination dysfunction. However, gene flow persists across chromosomal boundaries, mediated by individuals with intermediate karyotypes, maintaining species coherence. This suggests that while minor chromosomal differences are insufficient for reproductive isolation, accumulated CRs drive speciation over evolutionary timescales.

Studies indicate that gene flow in Carex is influenced by both geographic distance and karyotype rearrangements, impacting population genetic structure and species coalescence.

At the macroevolutionary level, recent advancements using the ChromoHiSSE model, which accounts for hidden states in chromosome evolution, reveal a complex relationship between dysploidy and speciation in Carex. While dysploidy does not universally increase speciation rates, it significantly drives diversification in specific clades. Notably, most CRs occur anagenetically, rather than cladogenetically, suggesting that single dysploidy events may not immediately induce reproductive isolation.

This observation supports the hypothesis that the unique nature of holocentric chromosomes allows for the accumulation of CRs over time, eventually establishing reproductive barriers. The "last-straw" hypothesis posits that a final dysploidy event triggers speciation after a threshold of CRs is reached. Alternatively, specific genomic rearrangement locations may determine evolutionary impact. These findings highlight the importance of holocentric chromosome dynamics in bridging micro- and macroevolutionary processes. By examining Carex, we gain crucial insights into how chromosomal evolution, particularly in non-canonical systems, shapes biodiversity.



Building upon a speciation theory to predict the link between microevolutionary processes and speciation time

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MEETING ROOM 114, August 19, 2025, 2:00 PM - 5:00 PM

Speciation – the process by which two lineages become reproductively isolated – plays a key role in the emergence and maintenance of biodiversity. Yet, our understanding of the time it takes for speciation to occur, and of the microevolutionary processes that influence this tempo, remain limited. For example, how mutation rates and population sizes influence speciation rates remain controversial. Here, we thoroughly characterize how population sizes, mutation rate and migration influence the time it takes to speciate under the hole adaptive landscape theory of reproductive isolation. We also characterize the relationship between genetic diversity and speciation rate. We find that increased mutation rates in general speed up speciation, as predicted by

the evolutionary speed hypothesis, but that the effect can be reversed if mutations are so frequent that outbreeding depression limits the accumulation of reproductive incompatibilities. The effect of population size depends on the speciation mode and the strength of local adaptation: if mutations causing incompatibilities are locally adaptive, we expect speciation to be faster in large populations, contrary to previous predictions. We test the obtained patterns with parameter values inferred using demographic speciation models on data from multiple plant species. Overall, our results shed light on the fundamental processes that modulate the speed at which new species emerge and establish a link between micro and macro-evolution.



Diverging selection on body size in specialist mammals

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MEETING ROOM 114, August 19, 2025, 2:00 PM - 5:00 PM

Body size is a fundamental organismal trait, related to a wide variety of physiological and ecological functions. Such relationships are often interacting and nonlinear, creating diversity of body size in a clade and complexity in the underlying mechanisms. In terrestrial mammals, larger bodies are associated with greater resource demand and higher dispersal ability. Here, we suggest that this trade-off is influenced by specialisation and propose a new hypothesis of diverging selection on body size for dietary specialists owing to efficiency in resource use, with supports from empirical patterns and a mathematical model. Compared to the overall proportion as a null expectation, our analysis of a global mammal species dataset found significantly lower proportions of specialists at intermediate body sizes, but higher proportions towards extreme sizes;

this pattern also applies to assemblages in major zoographic realms. Our mathematical model of competition between equal-sized specialists and generalists shows that specialists have higher equilibrium frequencies and are more likely to coexist with generalists, when they are at the extremes of the body size range than at intermediate sizes. The combined results strongly support dietary specialization as a key factor for shaping body size diversity. Our work highlights the value of connecting ecology and evolution in understanding the diversity of key traits like body size. Further investigations on the history of resource landscapes and lineage diversification dynamics should deepen our understanding of body size diversity in today's biota and help infer future changes as human modification of global habitats intensifies.



Diving depth and light environment influence countershading in pinnipeds

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MEETING ROOM 114, August 19, 2025, 2:00 PM - 5:00 PM

Countershading in animals is characterised by darker pigmentation on body regions that receive more illumination, while the opposite side is typically lighter, creating a dorsoventral luminance gradient. Although countershading has been extensively studied in terrestrial environments, its relationship with underwater light gradients, which predictably decrease with depth, remains unexplored. This study examines how diving depth, thermal environments, and body weight influence countershading variation among pinnipeds (seals, sea lions, and walruses) using phylogenetic comparative analyses. Our results indicate that mean diving depth and body weight

predict the degree of countershading, while the influence of thermal environments was less conclusive. Specifically, countershading decreases as diving depth increases, suggesting that light conditions have affected the evolution of countershading in pinnipeds. Additionally, body size had a stronger effect in males, with larger species exhibiting less countershading than smaller ones. This pattern may reflect the role of predation pressure in shaping countershading variation in males. Overall, this study highlights the importance of light environments and associated ecological pressures in the evolution of countershading in pinnipeds.



Evidence for Bergmann's and Allen's rules operating between species across the world's mammals

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MEETING ROOM 114, August 19, 2025, 2:00 PM - 5:00 PM

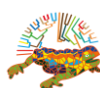
Background: Morphology varies substantially across the mammalian tree of life, from tiny shrews, giant whales, long weasels and tall giraffes. A common explanation for some of this variation invokes differences in thermoregulatory requirements. Sometimes known as Bergmann's and Allen's rules, these hypotheses describe the trend of animals being smaller (Bergmann's rule) and having larger extremities (Allen's rule) in warmer climates. These ecogeographic rules, however, are typically examined at small taxonomic scales (e.g., intraspecifically, or within genera or families), and thus it is unknown to what extent these relationships explain class-wide variation. Furthermore, alternative explanatory mechanisms to the temperature-morphology correlation have been proposed, notably a relationship with seasonal productivity. Here, using a global dataset of mammalian morphological measurements, we examine several versions of Bergmann's and Allen's rule across this wide taxonomic group, highlighting the potential role of thermoregulation in shaping vertebrate morphology.

Materials and Methods: To examine the potential relationship between temperature and mammalian morphology, we compiled a dataset of mammal body size, tail length, ear length, and hindfoot length for 4224 species. In a Bayesian phylogenetic framework, and after controlling for variation due to locomotory mode, we evaluated the global

and order-wide relationships between this morphological variation and three key climate variables (temperature, seasonality, and plant productivity).

Results: Bergmann's and Allen's rules generally hold across all mammals, with variation in temperature explaining morphological differences better than seasonality or plant productivity. In particular, Allen's rule holds in tails and hindfeet, but ear length shows no clear correlation with temperature. Furthermore, Allen's rule in tails and ears becomes more evident with increasing mass. Trends in both rules additionally show great variability between different mammalian orders.

Conclusions: These results suggest that thermoregulation, and especially selection for heat dissipation, is one force shaping the global variation in mammalian morphology. The strength of adherence to Bergmann's and Allen's rules varies across different taxonomic scales. This variation of strength is likely explained by the ecological and climatic niches of different clades, in conjunction with developmental biases, shaping the range of morphological variation at different phylogenetic scales. A more detailed understanding of the micro- and macro-evolutionary relationships between climate and morphology may thus help elucidate the future of mammalian populations in a warming world.



From micro to macroevolution: Evolvability predicts skull evolution across mammals

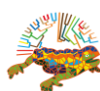
Rossoni D¹, Costa B², Penna A¹, Machado F³, Pavan A⁴, Assis A⁴, Zahn T⁴, Marroig G⁴, Houle D²

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MEETING ROOM 114, August 20, 2025, 10:30 AM - 12:30 PM

A pressing question in evolutionary biology is whether long-term evolutionary change is predictable from microevolutionary parameters. Simple theory suggests that directional selection may generate predictable responses, but evolvability, measured as standing variation, is expected to play a limited role over macroevolutionary timescales due to its transience and susceptibility to drift or constraint. Recent empirical findings, however, show striking correlations between evolvability and rates of morphological evolution. While such patterns are well-documented in insect wings and lizard limbs, it remains unclear whether similar predictability holds for rapidly evolving structures such as the mammalian skull. Here, we assess whether phenotypic variation can predict macroevolutionary divergence in mammalian skull morphology across four major clades: rodents, bats, carnivores, and primates. Using a dataset of 20 size-standardized linear cranial measurements and phylogenetic mixed models (MCMCglmm), we estimated evolutionary rates (R) and phenotypic variance (P) matrices across clades. We further estimated the slope of evolvability–divergence relationships along the phylogenies and explored whether species tend to diverge along multivariate phenotypic

directions associated with above-average evolvability. We find strong, positive correlations between within-clade P matrices and macroevolutionary rates, demonstrating that phenotypic variation reliably predicts evolutionary divergence within sub-families, families, and orders. Notably, while evolutionary changes in skull shape align with variation matrices, changes in size often deviate—suggesting distinct constraints or selective pressures on size. Furthermore, despite deep phylogenetic divergence (>80 Mya), we found that variation in one mammalian order significantly predict evolutionary rates in another, indicating the presence of evolutionarily conserved variance structures across orders. Our findings reinforce and expand the finding that deep, evolutionarily conserved patterns of phenotypic variation can shape macroevolutionary trajectories over tens of millions of years. These results call for a reevaluation of previous assumptions about the transient nature of variation and challenge the notion that directional selection alone governs long-term morphological change. While the causal mechanisms underlying this predictability remain elusive, our work suggests that the architecture of variation is a durable determinant of evolutionary potential—at large timescales.



From phylogenomics to population genomics: the role of geography versus ecology in driving *Coenonympha* butterflies diversification

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Understanding the role of geographical isolation and ecological adaptation in species diversification is a fundamental question in evolutionary biology. While allopatric speciation has been a paradigm for decades, growing evidences for porous boundaries between closely related species suggest that speciation may occur in the face of gene flow, driven by divergent selection along environmental gradients. The mixing–isolation–mixing (MIM) model of speciation has been proposed to explain mangrove divergence across the Malacca Strait, with cycles of isolation interspersed by periods of mixing, corresponding to sea level changes that have repeatedly opened and closed the migration between the Indian and Pacific Oceans. By permitting intermittent gene flow during speciation, thereby exposing genetic incompatibilities accumulated in allopatry to selection of pre-zygotic barriers (reinforcement), MIM cycles can potentially generate species at an exponential rate. Surprisingly, despite its power to explain the rapid build-up of reproductive barriers, the MIM hypothesis has not been extensively explored in other biogeographical contexts beyond its original application to South Asian mangroves.

In the Northern hemisphere, Quaternary climatic oscillations similarly opened and closed migration routes in the geological complex landscape of the Holarctic potentially resulting in a large increase in the number of species that form in areas where impermanent geographical barriers (e.g., glaciers intermittently melting, sea level changes) allowed recurrent cycles of isolation and mixing to occur. We explore the MIM hypothesis in one of the most species-rich and ecologically diverse butterfly genera of the temperate zone, the genus *Coenonympha* (Satyrinae, Nymphalidae) based on whole genome sequences from 32 species.

At the macroevolutionary scale, by combining phylogenomic, biogeographic, climatic niche and morphological trait evolution analyses, we

show that key episodes of species diversification coincided with periods of climatic change and biogeographic dispersal, yet these events do not appear to be associated with major shifts in climatic niche or wing morphology, consistent with a pattern of allopatric speciation. However, in the more recent evolutionary history (<3 million years), hindwing pattern evolution significantly deviates from neutral expectations, suggesting adaptive changes in this trait possibly linked to mate choice.

At the microevolutionary scale, focusing on two ecologically divergent sister species, the lowland pearly heath *C. arcania* and the highland alpine heath *C. gardetta*, we found little overlap between barrier loci (i.e., loci impermeable to gene flow) and loci under divergent selection which suggest that reproductive isolation and ecological adaptation are distinct mechanisms. A model of divergence in isolation with secondary contact (geographic speciation) was favored over a model of divergence with gene flow (ecological speciation) for these two species, as expected under the MIM hypothesis. This secondary contact produced a hybrid lineage that subsequently split in allopatry, giving rise to Darwin's heath *C. darwiniana* in Ticino (Switzerland) and *C. cephalidarwiniana* in the southern Alps with limited gene flow persisting between the hybrid lineages and their parental species. Beyond this alpine species complex, allopatric isolation and secondary gene flow appear to be pervasive in the genus, also involving the balkano-caucasian russian heath *C. leander*, the boreal scarce heath *C. hero*, and the two mediterranean insular endemics *C. corinna* and *C. elbana*.

In conclusion, both macroevolutionary and microevolutionary analyses support the view that alternating phases of geographic isolation and connectivity, as proposed by the MIM hypothesis, have been key drivers of speciation in this ecologically diverse butterfly genus.



Genetic constraints unaffected by selection predicts evolution within a fossil lineage

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MEETING ROOM 114, August 20, 2025, 10:30 AM - 12:30 PM

A growing body of research shows that evolution proceeds in directions of above-average population variance, or evolvability, over multiple generations and even across millions of years. Two non-mutually exclusive hypotheses explain this phenomenon: (1) genetic constraints funnels directions of phenotypic evolution and (2) selection aligns population variance in directions of phenotypic evolution. Most studies on contemporary taxa suggest, and show, that genetic constraints bias the direction of phenotypic evolution. However, it is difficult to disentangle and test the second hypotheses using contemporary taxa given the limited temporal scope. Therefore, it remains unknown if and the degree to which population variance changes over time, and if this is influenced by selection. Clonal organisms with a fossil record, such as bryozoans, provide a unique opportunity to branch micro- to macro-evolutionary studies as we can estimate genetic variation by measuring phenotypic traits. We leverage the 2.3-million-year fossil record of an extant bryozoan species, *Steginoporella magnifica*,

from the shores of New Zealand. We measured 6,779 zooids from 721 colonies to extract eight linear measurements. We then applied a microevolutionary framework at a macroevolutionary scale to test if population variance changes over time, if the direction of phenotypic evolution is biased towards directions of genetic constraints, and if selection re-aligns the direction of population variance. Additionally, we assess the validity of our fossil dataset by comparing it to contemporary populations. Our quantitative study finds that population variance changes through time, and, like previous studies, predicts directions of phenotypic evolution. Further, we find support for the genetic constraint hypothesis in shaping phenotypic evolution but not for selection in shaping population-variance. Finally, we find that the fossil record used provides a reasonable estimation of contemporary population variance. Future studies will apply this methodology to test if the role of population variance and selection during anagenesis (within-lineage) differs during periods of cladogenesis (speciation).



How reliable are phenotypic proxies of evolvability? Evaluating the relationships between P- and G-matrices across mammals

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MEETING ROOM 114, August 20, 2025, 10:30 AM - 12:30 PM

Quantitative genetic models have long emphasized the importance of the genetic variance-covariance matrix (G-matrix) for understanding the evolutionary potential of multivariate traits. However, for most taxa, especially non-model organisms, G is notoriously difficult to estimate, leading many to rely on the phenotypic variance-covariance matrix (P-matrix) as a proxy. Cheverud conjectured that for morphological traits, P matrices are both similar to G-matrices and much more accurately estimated, such that P-matrices may better represent evolvability than G-matrices. This conjecture has generally been supported by subsequent studies, resulting in the widespread use of P-matrices when good estimates of G are lacking. Here, we present the most comprehensive comparison to date of G- and P-matrices across mammals. Our dataset comprises G-matrix estimates for cranial traits in rodents, primates, and a marsupial, alongside P-matrices from over 400 mammal species, distributed across five major clades: bats, rodents, primates, marsupials, and carnivores. To characterize within-species variation, we estimated P-matrices for

samples in which each specimen was measured at least twice, enabling the separation of individual variation and measurement error. In addition, some traits were measured on both sides of the skull, enabling the estimation of asymmetry. All estimates of G and P were obtained using Bayesian mixed models implemented in MCMCglmm. Our results show that ignoring the influence of measurement error and asymmetry can significantly bias estimates of P. We then assess how well P captures evolutionarily relevant properties of G, including overall matrix structure, orientation, evolvability, conditional evolvability, and autonomy, accounting for sampling variation in both matrices. Our results highlight systematic biases in the use of P, some of which are clade-specific, and we identify conditions under which P may reliably approximate G. We also show that measurement error and directional asymmetry can significantly bias P-matrix estimates, emphasizing the need to account for these factors in comparative analyses. Our findings have broad implications for trait evolution studies, particularly in systems where G cannot be estimated directly.



Measuring the Macroevolutionary Adaptive Landscape

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MEETING ROOM 114, August 19, 2025, 2:00 PM - 5:00 PM

Despite the rising optimism that evolution at generational and geological time scales may be somewhat related, we lack a conceptual framework to study how selection in contemporary populations is linked to the pattern and process of adaptation inferred from phylogenetic comparative data. In my contribution, I present a new measurement theory for the study of phenotypic selection and adaptive landscape that unifies selection surfaces across multiple levels of biological organisation. Through an examination of various scenarios, I show that relativisation of fitness at the population level and the standardisation of traits to represent a natural logarithm of the ratio-scale are necessary to interpret contemporary selection estimates as a feature of the background selective regime (macroevolutionary adaptive landscape).

The propensity definition of a population will then be introduced as the key theoretical apparatus necessary for the unified concept of adaptive landscape. Subsequently, analyses of phenotypic selection on wing shape in two closely related pond damselflies: *Enallagma cyathigerum* and *Ischnura elegans* (Odonata: Coenagrionidae) will be presented to demonstrate how to operationalise the theory in empirical study systems. Estimates of phenotypic selection under the proposed measurement theory should, at least in principle, be comparable to corresponding parameters of Ornstein-Uhlenbeck processes fitted to phylogenetic comparative data. I will conclude by discussing the limitations of this framework and proposing possible solutions to address these limitations.



Merging evolutionary timescales to quantify adaptation

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MEETING ROOM 114, August 20, 2025, 10:30 AM - 12:30 PM

Positive diversifying selection promotes recurrent changes in amino acid sequences, driving adaptive evolution and helping evolutionary biologists understand the dynamics of species' adaptation. In phylogenetics, it is commonly inferred using the ratio of non-synonymous to synonymous substitution rates (dN/dS). Under the assumption that synonymous substitutions are neutral, dN/dS can inform us about the direction and strength of natural selection, and its widespread use in genomic studies highlights its importance. However, standard codon models typically rely on a single representative genome per species and neglect population-level processes such as selection on synonymous codons and GC-biased gene conversion (gBGC), both of which can bias dN/dS estimates and generate false positives, especially in the presence of within-species variation.

The growing availability of genome sequences in the genomics era makes it possible to study adaptive evolution with greater resolution by combining interspecific and intraspecific variation. In this study, we introduce a Polymorphism-aware Phylogenetic Model for Codon Evolution (PoMo-cod), which models adaptive evolution at a population genetics scale, while leveraging both fixed and polymorphic differences between species. PoMo-cod

advances codon modelling by integrating mutation biases, gBGC, synonymous codon and diversifying selection. We compare our framework with standard codon models in PAML - a widely used maximum likelihood method for detecting adaptive evolution - by simulating sequence evolution under PoMo-cod, mimicking great ape coding sequences. This comparison allowed us to assess how population-level forces bias signals of positive selection in dN/dS-based inference. We observed that in the absence of diversifying selection, but in the presence of confounding population forces, dN/dS estimates struggled to remain close to the neutral expectations and frequently produced inflated values. When explicitly simulating selection on non-synonymous sites, dN/dS recovered the expected signal and correlated well with the simulated values, but consistently overestimated the magnitude of selection. In conclusion, while dN/dS-based inference remains a valid and useful tool, it can overestimate adaptive signals when population-level processes influence sequence evolution beyond idealised assumptions. This study highlights current limitations in detecting diversifying selection and how population-aware approaches can improve the accuracy of evolutionary inference.



The diffused evolutionary dynamics of morphological novelty

Quintero I¹

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MEETING ROOM 114, August 19, 2025, 2:00 PM - 5:00 PM

Rates of evolution are fundamental to understand the processes that shaped the history of life. The predominant view holds that high rates of morphological evolution result from lineage transitions across peaks in an adaptive landscape, with subsequent slowdowns, but evidence remains debated. I developed a phylogenetic 'diffused Brownian motion' model that characterizes nuanced variations in evolutionary rates and use it to comprehensively assess body size evolution and its underlying rates for 2950 extinct and 792 extant species that span over 450 Mys of evolution. I find that evolutionary rates do not conform to expectations from

adaptive landscape theory, but rather have been stable, unaffected by the accumulation of morphological disparity. Long-term evolutionary trends, such as several net increases in clade-average body size, result both from sustained evolution at the lineage level and the sorting of species morphologies and their underlying evolutionary rates at the clade level, sometimes acting in opposite directions. These findings substantiate an active role of species on their environment that generate continuous novelty of lifeforms.



Within-population variance predicts evolution in a coccolithophore lineage transitioning between adaptive zones on the macroevolutionary adaptive landscape

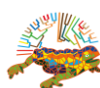
Voje K¹, Henderiks J²

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MEETING ROOM 114, August 19, 2025, 2:00 PM - 5:00 PM

Analyses of evolutionary time series of ancestor–descendant populations in the fossil record provide unique opportunities to explore key questions in evolutionary biology that are challenging to address by studying extant taxa alone. One example is whether our ability to predict evolutionary change using microevolutionary theory depends on the mode of evolution. We explore this by investigating multivariate phenotypic evolution of the well-sampled coccolithophore lineage *Reticulofenestra umbilicus* over a 12-million-year span. The temporal trait dynamics within this lineage can be separated into distinct and clearly delineated evolutionary modes, with two phases of stationary trait dynamics interrupted by a consistent trend toward larger sizes—culminating in a 75% increase

in size-related traits. Contrary to common assumptions about the correlation between tempo and mode in evolution, we find that phenotypic evolution occurs at the same rate, regardless of mode. We also demonstrate that phenotypic evolution occurs in directions of high evolvability—a microevolutionary parameter—both during the trend phase and the two stationary phases. This suggests that the predictive power of within-population variance on evolutionary trajectories is mode independent. We did not find any evidence supporting the idea that selection shapes within-population variance, which has been hypothesized to explain the commonly observed positive correlation between population variance and phenotypic divergence among living taxa.





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CONGRESS OF THE EUROPEAN SOCIETY FOR
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S37 - Museomics: Challenges and Possibilities



A legacy of low diversity: museum genomics uncovers persistent genomic erosion in the Mauritius kestrel

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POSTER SESSION 3 - P03.153, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Many species face the risk of population decline and genomic erosion—the accumulation of genetic load due to the loss of genetic diversity—which increases extinction risk even after populations recover from a bottleneck. Studies based solely on modern samples often have difficulties capturing genetic variation lost during population bottlenecks, limiting the understanding of the long-term genetic consequence. By integrating historical museum genomes, we can reconstruct lost diversity and assess the genomic consequences of population collapse more accurately.

The Mauritius kestrel (*Falco punctatus*) suffered one of the most extreme documented population bottlenecks—reduced to just four known individuals in 1974 with only one breeding female. Through intensive conservation management, the population rebounded to 350-400 individuals in the early 2000s and then declined again to <250 individuals. Here we analyze a whole-genome dataset comprising 15 historical specimens (2-16X) during the 19th century, and 59 modern samples (8-40X) from 1986 to 2021, along with genomes from six outbred and inbred sister species.

Demographic inferences reveal that the kestrel population was small, with N_e below 200, even prior to anthropogenic impacts associated with European colonization. Genome-wide heterozygosity has remained

low over the last two centuries, just 5% of that found in widespread kestrel species, reflecting its demographic history. The extreme bottlenecked resulted in a 30% loss of genome-wide diversity, leaving the Mauritius kestrel as one of the bird species with lowest genetic diversity worldwide. Runs of homozygosity (ROH, ≥ 1 Mb) constitute over 30% of the genomes of modern birds, a level three times as high as in the historical individuals. The fraction of long ROHs (>5Mb) further increased in recent times, indicating ongoing inbreeding within the recovered population. Compared to the nuclear genome, the mitochondrial genome experienced a drastic loss of genetic diversity reflecting the bottleneck, with only a single genotype found in recent (2012-2021). The level of genetic load had minimal fluctuations through time, with heterozygous load being already scarce in historical samples. The extremely low genetic diversity of the Mauritius kestrel through history may have buffered it from the effects of inbreeding depression through the bottleneck by reducing the deleterious alleles in heterozygous form. Yet, it also underscores the species' heightened vulnerability to future environmental and demographic challenges. Our results highlight how museum genomics provides the necessary historical context to understand long-term genomic erosion and inform conservation strategies.



Ancient genomic reconstruction of Late Pleistocene North America

Canis origins and distribution

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POSTER SESSION 3 - **P03.167**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

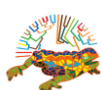
Background: The origins of red wolves (*Canis rufus*) and eastern wolves (e.g., *Canis lupus lycaon*, *Canis lycaon*, *Canis cf. lycaon*) have been debated for decades. Some argue that only two wild *Canis* species—grey wolves (*C. lupus*) and coyotes (*C. latrans*)—inhabit North America, and that both red and eastern wolves are recent hybrids between the two. Others suggest that *C. lycaon* represents a distinct North American lineage that includes both eastern and red wolves. Although a Pleistocene hybrid origin remains possible, little consideration has been given to their Pleistocene ancestors, such as the extinct Pleistocene coyotes (*C. l. orcutti*) and the Beringian wolves. Here, we analyzed genome-scale data of Pleistocene canids to compare against competing models of North American canid species origins and their distributions.

Material and Methods: Eleven genomes from Late Pleistocene *Canis* (nuclear and mitochondrial), dated between 39 and 14 calibrated thousand years before present (cal kyr BP), were analyzed from the Yukon Territory (Canada) and Tennessee (United States).

Results: Ancestral mitogenomes corresponding to *Canis latrans orcutti*, the wolf-like Pleistocene coyote, dated back to 223 cal kyr BP, with modern coyotes diverging in the Holocene, approximately 12 cal kyr BP. Embedded in this New World “*latrans*” clade were mitogenomes

associated with the highly debated red wolf (*C. rufus*) and eastern wolf (e.g., *Canis lupus lycaon*, *Canis lycaon*, *Canis cf. lycaon*), associating these taxonomic entities within the more wolf-like Pleistocene coyote (*C. l. orcutti*). Ancestral Pleistocene coyote mitogenomes were observed in Beringia (now Yukon) at 39 cal kyr BP, in modern California coyotes in western North America, and in 14 cal kyr BP samples from Tennessee—suggesting a western origin followed by eastward expansion of *C. l. orcutti*. Despite recent ancient DNA studies proposing that *C. lupus* was absent from North America south of the ice sheets during the Last Glacial Maximum—only colonizing the region following glacial retreat at the beginning of the Holocene—evidence from Mexican wolves and the 14 cal kyr BP Tennessee specimen in our study supports the presence and wider distribution of the ancient Beringian wolf during the Late Pleistocene. Nuclear whole genome analyses showed evidence of multiple introgression events over time between *C. l. orcutti* and ancient North American grey wolves.

Conclusion: Our findings support the need for temporal consideration when evaluating two- or three-species and hybridization models. We argue that the model begins with two Pleistocene species that subsequently gave rise to modern coyotes and a complex spectrum of introgressed wolf-like forms.



Ancient leaves, modern trees: Phylogenomics in *Amaranthaceae sensu stricto* using herbarium material

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POSTER SESSION 3 - **P03.152**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: The evolutionary history of *Amaranthaceae sensu stricto* has been shaped by multiple whole genome duplications and rapid radiations, which led to an ecologically diverse group of plants that includes many widespread species with a prominent potential to become a weed. Previous phylogenetic studies have struggled to resolve relationships within this clade, primarily due to limited genomic data and inadequate taxon sampling. As a result, the current classification of this economically important plant family remains unstable and fails to accurately reflect the evolutionary history of the lineage.

Materials and methods: To address these challenges, we designed a customised target enrichment bait set that targets 1,000 low-copy nuclear loci across the whole *Amaranthaceae s.s.* lineage. Leveraging the vast potential of global herbarium collections, we sampled nearly all known genera, covering 94% of genus-level diversity, including many rare or previously inaccessible taxa here for the first time. Phylogenetic reconstruction was conducted using both concatenation and coalescent-based approaches, with gene tree discordance, reticulation and gene duplication levels evaluated to understand the evolutionary history of this lineage and to identify the biological factors underlying the observed topologies.

Results: Our study presents a globally sampled and well-resolved phylogeny of *Amaranthaceae s.s.* Despite some herbarium material being over 160 years old, we recovered up to 98% of targeted loci, with an average recovery rate of 95% across samples.

While most nodes in the resulting species trees were statistically maximally supported, our quartet sampling analyses revealed high levels of gene tree discordance in the backbone of one of the major clades. Phylogenetic network analyses identified two reticulation events in this region: one associated with the origin of a species-poor genus, and another connecting the *Aervoids* and *Gomphrenoids*.

In addition to the three previously known whole genome duplication events in *Amaranthaceae s.s.*, we identified three further events. Two of these occur along the backbone of the phylogeny, and one is specific to the *Celosioid* clade.

Importantly, our results challenge the current morphological framework used for tribe classification. Many traditionally used characters are shown to be homoplasious and phylogenetically uninformative. Based on molecular evidence, we therefore propose an updated taxonomic framework in two subfamilies and nine tribes.

Conclusions: Our analyses highlight the power of herbarium-based target capture approaches for resolving complex evolutionary histories, even in morphologically challenging and genetically complex groups like *Amaranthaceae s.s.* We illustrate here the immense importance of historical collections and how they can be utilised to answer modern questions. Our phylogeny lays the groundwork for future studies on trait evolution, biogeography, and conservation priorities across the lineage and provides a scalable framework applicable to other taxonomically challenging and poorly sampled plant lineages.



Developing an accessible multi-locus barcoding approach for the field and museum

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POSTER SESSION 3 - **P03.168**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Biodiversity has declined between 2% and 10% in the past century, and an estimated 1 million species face extinction, yet only a fraction of species are known to science. By documenting and archiving this diversity and its loss, museums and their collections play a major role in understanding the impact of the current biodiversity crisis. One of the greatest challenges doing so, is generating a complete inventory of the earth's species. In this quest of inventorying, molecular barcoding has become one of the standard tools for species identification. However, the use of single mitochondrial genes is associated with a range of biases from e.g. Incomplete Lineage Sorting, hybridization, NUMTs, Wolbachia infections, or simply missing information content. Today, massive multi locus approaches, such as RADseq, UCEs or Whole Genome Sequencing, have been proposed alleviate these issues. Although they provide unprecedented insights into the biology of a species, they require comparably high effort, special expertise, and sufficient funding. Although they build the backbones of modern biodiversity research, funding to maintain, build and examine collections is scarce. This impediment as well as the rate of the current biodiversity loss create the need for robust molecular approaches that can be used with fresh as well as museum samples and are simple, accessible and fast.

Materials and methods: We created a bioinformatic pipeline which uses orthologous sequences from selected genomes to find novel, standardized taxonomic markers. The pipeline first identifies candidates with the necessary marker structure and calculates their phylogenetic signal and ranks them by this

measure. Using this ranking, one can select the most promising markers for testing in the laboratory. The markers can be amplified by Polymerase Chain Reaction (PCR), which provides a simple and cost-efficient workflow.

We used this pipeline to generate a standardized nuclear marker set for use in spider taxonomy. To do so, we first assembled a spider genome dataset consisting of 31 spider genomes and 4 outgroups spanning the whole spider tree of life. This dataset was used to identify orthologous regions by whole genome alignment and subsequent HMMER searches, before running the pipeline to identify candidate markers. Then, the marker set was tested with a comprehensive selection of spider specimens. The Barcodes were amplified using PCR and sequenced on a Nanopore Flongle. We examined the power of the marker set for species delimitation and phylogeny reconstruction.

Results: Here, we present the pipeline which enables the generation of standardized barcode sets for any given taxon. We generate a standardized marker set for spiders and show that, despite having few markers compared to massive multi-locus approaches, it has the power for robust phylogenetic inference and species delimitation.

Conclusions: We built a pipeline which can suggest novel taxonomic markers which on the one hand provide significantly increased power, on the other enable a fast, simple and affordable workflow. As funding for museums and collections is scarce, the novel approach and markers provide a good tradeoff between number of analyzed loci, and accessibility and affordability.



DNAharvester: A Nextflow Pipeline for Processing and Analyzing Highly Degraded DNA from Ancient and Historical Specimens

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POSTER SESSION 3 - P03.164, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Ancient DNA (aDNA) studies increasingly rely on robust bioinformatic pipelines to process highly degraded sequencing data from archaeological and museum specimens. Here, we present DNAharvester, a highly scalable and state-of-the-art Nextflow pipeline designed to process and analyze sequencing data from such challenging samples. The pipeline accepts raw single- or paired-end FASTQ files, performs adapter trimming and read merging with Fastp, and align reads to a reference genome using bwa aln with parameters optimized for aDNA. To remove contaminant (non-endogenous) reads, the pipeline includes negative filtering using a Kraken2 database, as well as, supports competitive mapping, where reads are aligned to a reference that includes both target and decoy sequences (e.g., human, bacterial, viral). Only reads that uniquely map to the target sequences are retained. DNAharvester integrates AMBER to identify the minimum read length per library that maximizes usable data while minimizing spurious alignments. For screening datasets, it estimates library complexity and predicts sequencing yield using Preseq. The pipeline identifies repetitive elements in the reference using RepeatModeler and

RepeatMasker, and detects CpG sites, generating a BED file with both repeats and CpG regions masked. PCR duplicates are removed both per-library and in the case of multiple libraries per sample, from merged bam files per sample to eliminate PCR and strand duplicates. Variants are called in both genotype likelihood and pseudo-haploid frameworks (via random allele sampling) using ANGSD, enabling downstream population and phylogenetic analyses. The pipeline also compiles key statistics using tools such as FastQC, MultiQC, AMBER, Samtools, and custom scripts, reporting metrics for each library in a CSV file.

DNAharvester requires no programming experience and includes detailed documentation for all steps, ensuring accessibility to users across disciplines. Implemented in Nextflow with support for Conda and container environments, the pipeline ensures full reproducibility across computing systems. By standardizing aDNA processing, DNAharvester enables robust genomic analysis of ancient and historical specimens and contributes to the growing field of museomics. The pipeline will soon be available on GitHub.



From almost extinct to rescued? Temporal dynamics of genomic erosion after demographic recovery.

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POSTER SESSION 3 - **P03.148**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Many species that suffer from population decline are at higher risk of genetic drift and inbreeding, which results in a loss of genetic diversity, eventually threatening their survival. These events can be documented by comparing the genomes of historical samples pre-dating their bottleneck and modern populations. Integrating in-situ (wild) and ex-situ (captive) conservation efforts can mitigate this genetic diversity loss and help prevent extinction of endangered wild populations.

One exceptional case is the whooping crane (*Grus americana*). This endangered species experienced severe population declines beginning in the 18th century which culminated in a collapse to ~20 individuals by 1944. Subsequent legal protections and conservation actions (including captive breeding) have since increased the population from a stock of 16 individuals to approximately 840. Such a success has opened the debate to whether this species should no longer be classified as endangered by the IUCN Red List and the U.S. Endangered Species Act. However, given the severity of the bottleneck, the impact on genomic diversity for the future survival of the species remained unclear.

To assess the temporal dynamics of genomic erosion in this species, we generated a high-quality reference genome, and re-sequenced and analyzed 16 historical (dated before the population collapse) and 37 modern genomes, including wild individuals and four generations of captive-bred individuals.

Genomic demographic reconstructions reveal a steady decline, accelerating over the past 300 years with the European settlement of North America. We show that despite demographic recovery, the species has lost 70% of its genetic diversity. Although the modern population bottleneck reduced the ancestral genetic load, its realised load has increased. In fact, the modern populations possess more realized load than masked load, which is rarely observed in wild populations. Although this could make the species more resilient to subsequent inbreeding, it possibly also results in a chronic loss of fitness.

The whooping crane breeding program is based on a highly detailed and well-curated studbook. By taking advantage of such a resource, we integrated pedigree and genomic data to underscore the key role of breeding management in reducing recent inbreeding. Despite this effort, ongoing heterozygosity loss, load accumulation, and background inbreeding argues against the species' downlisting from their current endangered status. Finally, in an attempt to rescue genetic diversity, we described the presence of private genetic variation in wild and captive populations. This suggests that wild-captive crosses could enhance genetic diversity and reduce the realized load.

The findings presented here emphasize the key role of temporal genomics in informing conservation management and policy.



Genomic evidence for West Antarctic Ice Sheet collapse during the Last Interglacial

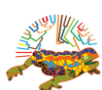
Lau S¹, Wilson N², Golledge N³, Naish T³, Watts P⁴, Silva C⁵, Cooke I¹, Allcock L⁶, Mark F⁷, Linse K⁸, Strugnell J¹

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POSTER SESSION 3 - P03.151, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The marine-based West Antarctic Ice Sheet (WAIS) is considered vulnerable to irreversible collapse under future climate trajectories, and its tipping point may lie within the mitigated warming scenarios of 1.5° to 2°C of the United Nations Paris Agreement. Knowledge of ice loss during similarly warm past climates could resolve this uncertainty, including the Last Interglacial when global sea levels were 5 to 10 meters higher than today and global average temperatures were 0.5° to 1.5°C warmer than preindustrial levels. Using a

panel of genome-wide, single-nucleotide polymorphisms (SNPs) of a circum-Antarctic octopus, we show persistent, historic signals of gene flow only possible with complete WAIS collapse. All octopus specimens had been stored in museum/research collections for 30+ years and target capture sequencing was used to retrieve SNPs. Our results provide the first empirical evidence that the tipping point of WAIS loss could be reached even under stringent climate mitigation scenarios.



Genomic insight into the population history of Norwegian lemmings

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POSTER SESSION 3 - P03.163, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The Norwegian lemming (*Lemmus lemmus*) is a small rodent endemic to the Fennoscandian mountain tundra. The species likely evolved during the Late Pleistocene, although its exact origins remain unclear. Interestingly, unlike other Fennoscandian mammals, it has been suggested that the Norwegian lemming did not recolonise Fennoscandia from a southern refugium after the last Ice Age. Instead, previous research based on short mitochondrial markers has proposed that the species may have survived the Last Glacial Maximum in a local ice-free refugium, from which it subsequently expanded. Furthermore, short genetic markers have revealed a genetic gradient but no clear structure across the modern-day

distribution, with the exception of the Pallasjärvi sub-population. To further investigate the population history of the Norwegian lemming, we sequenced and analysed the complete genomes of 91 modern *Lemmus lemmus* and eight ancient lemmings from the genus *Lemmus*. We reconstruct the phylogenies and population structure of *Lemmus lemmus* to examine the modern-day population patterns and differentiation across sub-regions. Additionally, we investigate the genetic relationship between the species and ancient lemmings from Western and Eastern Europe, to gain further insight into the species origin. Finally, we present a new, EBP-quality *Lemmus lemmus* reference genome.



Genomic Insights into Biodiversity Decline: Integrating Reference Genomes and Population Genomics within the BIGFOOT Project

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POSTER SESSION 3 - P03.149, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The accelerating global decline in biodiversity underscores the urgent need for refined conservation strategies. While traditional approaches emphasize species monitoring and habitat protection, genomic data can reveal early signatures of population decline—such as reduced heterozygosity, decreased effective population sizes, elevated inbreeding, and increased mutational load—long before demographic declines are detectable. Despite this potential, most conservation genomic studies have centered on a narrow subset of flagship or model species, limiting our understanding of genetic erosion across the majority of taxa.

As part of the BIGFOOT (Biodiversity Decline's Genomic Footprint) project, we investigate the genomic impacts of population decline in ten threatened and

often overlooked species including insects, vertebrates, and mollusks. Despite the advancements in sequencing technology, most organisms still lack publicly available high-quality chromosome-level genomes, with even fewer annotated in detail. Here we focus on generating such genome assemblies for five insect species from the orders Coleoptera, Orthoptera, Odonata and Lepidoptera, using long-read sequencing and Hi-C technologies. By combining reference genomes with whole-genome data from modern and historical (museomic) samples, we explore how species respond at the genomic level to both environmental and anthropogenic pressures. This comparative approach enables us to evaluate how genomic landscapes differ across taxa and over time, revealing underlying patterns of population decline.



Harnessing a Nearly Complete Herbarium Collection to Unravel the Evolutionary Radiation of Astragalus (Fabaceae)

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POSTER SESSION 3 - **P03.165**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Astragalus (Fabaceae, Papilionoideae), comprising over 3100 species, is widely recognized as the largest genus of flowering plants. It represents a striking case of recent and rapid evolutionary radiation, particularly in arid and semi-arid regions of the Northern Hemisphere. Despite its diversity and ecological success, our understanding of the evolutionary drivers and adaptive traits underpinning this mega-radiation remains fragmentary. This is primarily due to limited taxon sampling and poorly resolved phylogenetic relationships, which have hampered comparative analyses across the genus.

A leading hypothesis suggests that edaphic specialization and the colonization of extreme or isolated microhabitats have played a major role in the divergence and radiation of *Astragalus* lineages. We are constructing a robust phylogenomic framework for the genus using a target enrichment approach to test this and other evolutionary hypotheses. We developed a novel bait set targeting 686 orthologous genes (819 exons), specifically designed for the Astragalean clade. This tailored approach enables us to resolve deep and shallow relationships across the genus with high accuracy, even among morphologically and ecologically disparate taxa.

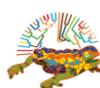
Our sampling strategy focuses on the main centres of *Astragalus* diversity – especially the Irano-Turanian floristic region, which hosts more than 1500 species – and is built upon the exceptional holdings of the *Astragalus* herbarium collection at the Botanical State Collection Munich (BSM-SNSB). This collection includes approximately 25000 specimens, representing global diversity and encompassing material from regions that are now difficult or impossible to access due to political conflict, particularly Afghanistan.

Many of these specimens serve as invaluable historical vouchers for taxa that are otherwise underrepresented or absent from molecular studies. Remarkably, we extracted usable genomic DNA from specimens up to 115 years old.

We expanded our dataset by integrating publicly available genomic resources, including genome skimming, transcriptomes, and earlier target enrichment data. This allowed us to build the most comprehensive and robust phylogeny of Old World *Astragalus*. Our analyses recovered a well-supported tree at the subgenus level, broadly consistent with earlier studies, but also revealed notable differences in sectional delimitations and strong signals of gene tree discordance, cyto-nuclear conflict, and potential reticulate evolution. These findings point to a more complex evolutionary history than previously appreciated in the genus.

In parallel with the phylogenomic work, we developed a scalable workflow for digitizing the *Astragalus* herbarium collection at BSM-SNSB. This includes high-resolution imaging and automated extraction of text-label data. Using machine learning techniques and large language models, we retrieved occurrence data directly from specimen images, allowing us to integrate ecological and geographic information with genomic data.

Our integrative approach highlights the power of combining museomic resources with cutting-edge phylogenomics to unravel complex evolutionary radiations in hyperdiverse plant groups. By leveraging the depth and historical reach of herbarium collections alongside genomic and ecological data, we are gaining new insights into the mechanisms driving diversification in *Astragalus*, including ecological specialization, morphological evolution, and biogeographic history.



Limits and best practices for local ancestry inference in imputed ancient genomes

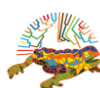
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POSTER SESSION 3 - P03.161, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Recent advances in imputation of ancient genomes have created the opportunity to apply state-of-the-art methods of statistical genetics to ancient genomes of different species to study various aspects of their evolutionary histories across large timescales. One popular class of such methods, known as local ancestry inference (LAI), aims to “paint” an individual’s genome as a mosaic of segments inherited from ancestral source populations. Despite their great potential to reveal patterns of genetic ancestry across the genome, most LAI methods have been designed to study relatively recent admixture events specifically in human history, using large panels of present-day genomes. However, it is currently unknown how reliable LAI is in ancient DNA datasets, which are characterized by scarce geographic and temporal sampling, various degrees of divergence between ancient source proxies

and admixing populations, and their complex demographic histories. In this study, using genetic simulations within the slendr framework, we evaluated the performance of four LAI methods, RFMix, FLARE, MOSAIC and simplAI, together with a popular admixture dating software DATES, under different demographic scenarios, various temporal sampling schemes and sample sizes, as well as admixture dates. We quantified their accuracy as a function of these parameters, and tested to what extent does inclusion of source proxies pre- or postdating the admixture event or grouping putative sources from different time points, affect the inferences. Our study establishes the limits of LAI in ancient genomes and provides general guidelines for LAI in future studies, which we demonstrate by a proof-of-concept LAI in a set of ancient human genomes.



Museomic challenges to delimit the nine-banded armadillo species complex

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POSTER SESSION 3 - P03.147, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The nine-banded armadillo (*Dasypus novemcinctus*) is the most widespread xenarthran species across the Americas. Recent studies have suggested it is composed of four morphologically and genetically distinct lineages of uncertain taxonomic status. To address this issue, we used a museomic approach to sequence 80 complete mitogenomes and capture 997 nuclear loci for 71 *Dasypus* individuals sampled across the entire distribution. Museum specimens with postmortem mutations and low levels of endogenous DNA are particularly prone to artifactual substitutions and cross-contamination. Incorrect genotype calls are expected to have a significant impact on population genetic analyses, in particular by leading to inaccurate conclusions about genetic diversity and population structure. Some of these errors, such as cross-contamination,

could even mimic gene flow, which is particularly problematic for species delimitation. Appropriate data cleaning should improve species delineations but disentangling errors from biological signals of introgression remains a challenge. For this reason, we implemented methods to detect cross-contamination based on mitochondrial read depth and carefully cleaned reads from sequencing and contamination errors. We reconstructed phylogenetic relationships and used multiple approaches to delimit species and assess genetic exchange. This provided a trustfull understanding of speciation events and allowed us to recognize four species (*D. novemcinctus*, *D. fenestratus*, *D. mexicanus*, and *D. guianensis*), including a new species from the Guiana Shield, which is the first new armadillo species described in the last 30 years.



Museomics reveals the true extend of horizontal transfer in *Drosophila*

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POSTER SESSION 3 - P03.154, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

When we compared the genomes of 200-year-old fruit flies (*Drosophila melanogaster*) from museum collections and recently captured wild flies we were surprised to find that large parts of the fruit fly genome were missing from the old specimens. About 1 million base pairs, roughly 1% of the genome, were absent from the old flies. A detailed analysis revealed that the missing parts largely consist of transposable elements (TEs) - parasitic DNA sequences that replicate in genomes.

These TEs can occasionally be horizontally transferred (HT) between species by an unknown mechanism. Traditional approaches, such as comparing the genomes of different species, suggest that HT is a rare event. However these

approaches suffer from several limitations and thus underestimate the true extent of HT. Museomics enables an alternative approach: by comparing the genomes of old and extant specimens we can for the very first time obtain an unbiased estimate of the true extent of HT.

Our work showed that in total 12 different TEs have invaded the fruit fly genome over the last 200 years, suggesting that the extent of HT in insects may be massive. This finding raises a lot of questions, such as whether other species show a similarly high rate of genomic invasions. To address these questions we are launching a collaborative effort to sequence the genomes of many historical specimens from different *Drosophilidae*.



Past and future anthropogenic impacts on Atlantic bluefin tuna through the eyes of demographic and adaptation analyses on range-wide whole genomes

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POSTER SESSION 3 - P03.169, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Atlantic bluefin tuna (*Thunnus thynnus*) have been commercially exploited for centuries, yet historical baselines used in fisheries management may underestimate the extent of past declines. Recent genomic analyses suggest that ABFT underwent a demographic decline as early as 1900, long before modern fisheries records indicate. This decline coincided with a reduction in genome-wide diversity, suggesting potential long-term consequences for population resilience. High coverage resequencing of 45 modern and 45 ancient specimens (dating back to 3000 BC) collected across the species range, from the Mediterranean to the Gulf of Mexico, was undertaken to improve

resolution in detecting genetic erosion and adaptive change, capitalizing the recently released chromosome level and annotated *Thunnus thynnus* genome. By integrating genomic, environmental and fisheries data, this research will provide a deeper understanding of ABFT population structure, historical demography and resilience. This knowledge can improve stock assessments, refine recovery targets and inform long-term conservation strategies, ensuring that management decisions take into account both historical and current population dynamics.



Reconstructing the evolutionary history of the Tyto owls' diversification through museomics

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POSTER SESSION 3 - P03.158, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Museum collections are a crucial source of past biological and genetic information, allowing the identification of key adaptative, demographic and ecological events throughout the evolutionary history of species. However, depending on their preservation method and collection time, DNA from old specimens can suffer from degradation and/or contamination that limits the quality of the genetic information we can retrieve. Nonetheless, several evolutionary research questions require the sampling of many individuals covering large geographical and temporal ranges, which would not be possible without museum collections. The genus *Tyto* –commonly known as barn owls– are an excellent example of a diverse and widely distributed group of predatory birds, covering every continent except Antarctica. Therefore, given their wide geographic range, we used museum collections from around the world to obtain a large genomic dataset with the overall aim of evaluating the species phylogenetic relationships and the evolutionary processes that led to their speciation. Today, 17 species are recognized to form the genus *Tyto* and although their ecology and taxonomy have been broadly studied, the most recent phylogenetic reconstruction of the genus (Uva et al., 2018) is based on very few molecular markers. While this phylogeny brought light to crucial questions on the phylogenetic relationships, it fails to solve several relationships. Interestingly, most of the species in unresolved clades belong to the Core *Tyto* group, a group presenting the largest genetic diversity across the *Tyto* genus.

To disentangle the evolutionary history of the barn owls and the processes that could have contributed to their diversification, we use >100 museum specimens across 15 species, making this the most complete genomic study on the phylogeny of the genus *Tyto*.

Our results show that species trees estimated from whole mito- and nuclear genomes present similar

phylogenetic relationships among deeper branches, supporting previous studies. For instance, results suggest an early divergence of two main clades (Core-*Tyto* and the Common-Barn-Owl groups) in the late Miocene (~ 12Mya) and confirms the monophyletic status of several species like *T. alba*, *T. furcata*, *T. javanica* and *T. novahollandiae*. Furthermore, our whole-genome approach resolves the monophyletic status of sooty and grass owls previously found as paraphyletic. Nuclear genome results also suggest new positions on the phylogenetic tree for a few poorly studied species (e.g *T. aurantia*).

Several widely used taxonomic classifications at the subspecies level are not supported by the whole-genome data. For instance, subspecies of *T. alba*, despite their morphological differentiation, do not show any distinctive clustering, suggesting this classification do not correspond to divergent genomic lineages but rather the occurrence of large morphological diversity at the species level. We also identified ancient hybridisation events involving species from the Core *Tyto* owl cluster, consistent with their higher genetic diversity and demographic patterns variation through time. Interestingly, this group of species is mainly distributed in Austral-Asia and areas around Wallacea, a widely recognized hotspot of biodiversity, with a high degree of endemism.

Finally, using ancestral recombination graphs (ARGs) we estimated historic geographic dispersal patterns of ancestors aiming to elucidate the temporal framework of important evolutionary events on the speciation of this clade.

The diversification of *Tyto* species presents an intricate evolutionary path, only possible to resolve by using whole-genome approaches. In this study we showcase the importance that museum collections have for reconstructing complex evolutionary histories, despite the challenges that degraded DNA can present.



Shelf Life: Optimizing Ancient DNA Recovery from Museum-Curated Pleistocene Specimens

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POSTER SESSION 3 - P03.160, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Recovering ancient DNA (aDNA) from ancient specimens often requires tailored laboratory approaches, and this is especially true when working with material that may have been housed in museum collections, due to possible glue and consolidant treatments and frequent handling. Here, we present an optimized protocol that significantly improved DNA recovery from a set of ~30,000-year-old mammoth sub-fossils, some of which have been curated in museum collections for over 100 years.

We conducted two key comparisons to evaluate the impact of pre-extraction and damage-repair treatments. First, we compared a traditional drilling-based approach without bleach or predigestion to a crushing method using a mortar and pestle, followed by a 4-minute bleach treatment. Second, we assessed the effects of three USER enzyme damage-repair strategies: 3-hour, 30-minute, and no treatment. The

protocol optimization was facilitated by the use of a plate-based library preparation method (Gilardet et al 2025), which allowed us to prepare 84 libraries (six per individual) in a single batch, minimizing batch effects and enabling robust cross-treatment comparisons. Our findings demonstrate the benefits of a bleach pre-treatment in recovering DNA from these materials, as it significantly reduces DNA from non-target organisms. The USER enzyme treatment conditions had less of an impact on DNA recovery, indicating that partial and full USER treatments are feasible for material of this antiquity. We emphasize the importance of flexible, context-sensitive methods when working with ancient material. These optimizations have broad relevance for improving aDNA yields from museum-held collections, expanding the scope of specimens that can be included in genetic analyses of the past.



Speciation around a mountain chain: Hybridisation dynamics in a group of lek-mating Birds-of-paradise (Paradisaea)

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POSTER SESSION 3 - **P03.156**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Speciation is a continuous process and understanding how incipient lineages evolve along this continuum remains a major challenge. Hybridisation and sexual selection play key roles in driving or constraining speciation, but their interaction can be complex. While strong mate choice should limit gene flow, interspecific hybridisation can lead to either lineage fusion or the introduction of genetic variants that can facilitate diversification. Birds-of-paradise provide a compelling system in which to explore these dynamics, as they exhibit some of the most elaborate forms of mate choice and sexually selected traits, and yet commonly hybridise. Using a large whole-genome dataset from 108 historical specimens, we investigated phylogenetic relationships, patterns of gene flow, and the genetic differentiation across the genus *Paradisaea* and its sister genus *Paradisornis*. Our results reveal species that vary in their relative position along the speciation continuum. While some allopatric species exhibit strong genetic differentiation, other species admix extensively even though they differ in plumage traits. The widespread mainland species complex (*P. minor*, *P. apoda*, and *P. raggiana*) in particular, follows a geographic gradient of genetic exchange, forming a pattern in which geographically close populations show increasing

admixture. Notwithstanding the presence of phenotypic variation in putatively sexually selected traits, the gradual shift in shared ancestry raises questions about the extent to which these taxa represent distinct species, or rather, populations along a broader continuum of differentiation. Most strikingly, despite consistent signatures of genomic admixture between contiguous populations, *P. minor* and *P. raggiana* appear to be largely reproductively isolated in eastern New Guinea where the two lineages meet at the end of the central mountain range. The spatial distribution of divergence and hybridisation between *P. minor*, *P. apoda*, and *P. raggiana*, is therefore ring-like along the New Guinea highlands and presents a prime example of incipient lineages at different stages along the speciation continuum. Our findings illustrate how speciation can be simultaneously shaped by a complex interplay of geography, gene flow, and sexual selection, and how such processes in concert can lead to different stages of reproductive isolation across lineages and regions. Furthermore, this study demonstrates the value of historical museum collections for genomic studies of speciation dynamics, especially for species and populations that are difficult to sample in the wild.



The Genomic Revolution and Its Limitations: A Summary of Genomic Data Available for Eukaryotic Species

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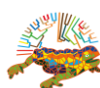
POSTER SESSION 3 - **P03.166**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: In recent decades biodiversity research has been transformed by advances in genomics. Reduction of sequencing cost and improvement of sequencing platforms have enabled large scale initiatives aiming to provide reference-quality genomes for all eukaryotic species. This includes landmark projects such as the Earth BioGenome Project, the Vertebrate Genome Project, and the Darwin Tree of life. While these efforts have generated hundreds of high-quality genomes, many clades and ecosystems remain invisible at the genomic level, limiting our ability to investigate fundamental evolutionary questions and inform conservation strategies. Here, we explore the current landscape of genomic data across eukaryotic species and how museum samples can provide further data to address the existing limitations.

Materials and Methods: We compiled a dataset of available reference genomes from major genomic consortia by accessing genome assembly records from the NCBI Genomes database, focusing on plants and animals. Species-level geographic distribution data for GenBank-sequenced

taxa were retrieved from the Global Biodiversity Information Facility (GBIF) and visualized by country and ecosystem at species and genus level. In parallel, we obtained museum specimen occurrence data from the Natural History Museum, London (via GBIF), and mapped it to assess how these archived specimens could complement existing genomic resources.

Results & Conclusions: Despite the recent advancements of genomics revolutionising our ability to explore biodiversity, genomic resources appear to be disproportionately concentrated from species from temperate zones, economically important taxa, and lineages with established research infrastructure. This contrasts species from tropical biomes, as well as invertebrates and plants, which remain poorly represented despite their overwhelming contribution to global biodiversity. The billions of specimens collected over the centuries in historical collections provide an opportunity to bridge these taxonomic, geographical and temporal gaps.



Ancient Genomes, Modern Climates: Tracking Alpine Plant Changes Through Herbarium Records

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Alpine plants are highly vulnerable to the effects of climate change, as temperatures in alpine regions have risen at twice the global average over the past two centuries. These plants, adapted to cold climates, face the dual threats of habitat loss and genetic erosion. While recent studies focus on distributional shifts and phenological changes, the genetic consequences of these challenges remain less explored.

In this study we utilized herbarium specimens to investigate the past and present genomic diversity of an alpine plant endemic, *Favratia zoysii*, and compared ancient DNA from historical records with data from present day populations. By generating a genomic timeline spanning from 1780 to 2023, we establish a baseline of genetic diversity for this species before the industrial-era climate shifts. Using an Illumina short-read approach on specimens from multiple European herbaria, we assessed allele frequency changes over time to identify signs of adaptation and genetic erosion. Specifically, we aimed to determine whether the species has successfully adapted to climate fluctuations, pinpointing the genomic regions involved, or if it exhibits

allele loss and declining genetic diversity, signaling potential vulnerability.

One challenge of this study was selecting species well represented in herbaria while ensuring their historical collections overlap with current populations. To address this, we developed a novel index that evaluates all alpine species (but can be used on any other geographical range) based on herbarium record density and spatial-temporal overlap with modern occurrences. By integrating digitized herbaria and biodiversity databases (e.g., GBIF), this index ranks species suitability for comparative genomics, identifying those with extensive historical collections and present-day overlap.

This work highlights the power of museomics in tracing evolutionary trajectories, assessing climate-driven genomic changes in alpine plants, and guiding conservation. By combining ancient DNA with modern DNA, we demonstrate the value of herbarium collections for monitoring biodiversity in a changing world.

Keywords:

herbarium genomics, alpine plants, genetic diversity, climate change, museomics



Combining eDNA and Museomics to Enhance Biodiversity Monitoring

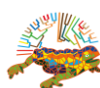
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Assessing the distribution of rare marine species can be challenging due to their inherent low abundance and limited occurrence. Considering their elusive nature, traditional monitoring methods can be inefficient, destructive and resource intensive. We thus require more specialised survey techniques to map their distribution accurately, which is essential for targeted conservation efforts and understanding the potential impact of environmental changes. Environmental DNA (eDNA) is a powerful and non-invasive tool and has proven efficient in detecting even rare species, notably in marine environments. However, to achieve effective eDNA monitoring, we require a comprehensive DNA barcode reference

database, which should include all the species of interest. To improve the DNA reference database and improve species detection, we sequenced whole mitochondrial genome of hundreds of fish specimens from ichthyological collections across Europe using specific protocols dedicated to museum samples. We optimised lab protocols to extract and sequence DNA from samples preserved in formaldehyde – a commonly used tissue preservative known to damage DNA. The DNA sequences were added to the eDNA reference database to improve species distribution for endangered fish species and detect potential diversity hotspots.



Harnessing the potential of historical DNA in population genomic studies on insects - a case study

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The onset of industrialization marked the beginning of rapid and extreme anthropogenic global change. Agricultural change, in particular, including changes in land use, increased use of pesticides and fertilizers, and the introduction of invasive species, has caused a biodiversity crisis strongly affecting insects. Accumulating evidence is showing strong declines of insect biomass, shifts in species composition, and extinctions, all of which are threatening key ecosystem services like pollination. Unfortunately, studies on the decline of insects are often limited to spatial comparisons or to relatively short time frames. A promising approach to overcoming these constraints is to perform temporal comparisons using genomic data from specimens stored in museum collections.

The common carder bee, *Bombus pascuorum*, is an important pollinator. While widespread in Sweden, there has been evidence of a decline in their relative abundance since the 1960s. To investigate the possible genomic consequences of this decline, we performed whole-genome sequencing on dry-pinned historical samples (~100), collected over the course of the past 150 years throughout Sweden. Our dataset was complemented with genomic data from modern specimens as well as previously published modern genomic data (~60). We performed population genomics analyses to identify temporal changes in population structure and investigated

possible changes in genomic diversity over time.

The historical samples yielded very short read lengths (mean read length: average 58.3 bp, range 41.8 - 89.0 bp), which correlated with sample age, indicating that DNA fragmentation happens at a relatively constant rate over time. However, the endogenous content in these samples was high (average 81%), which allowed us to generate low-coverage genomes (average 3.5x). Our population genomics results indicate the existence of three differentiated *B. pascuorum* populations located on the island Gotland, in the Lappland mountains, and the rest of Sweden, respectively. These populations roughly correlate with the described subspecies (Gotland - ssp. *gotlandicus*, Lappland mountains - ssp. *smithianus*, rest of Sweden - ssp. *pallidofacies* and *sparreanus*). This temporal dataset indicates that, despite the reported declines, both population structure and genomic diversity have been remarkably stable throughout the last 150 years.

We find that the genomic integrity of *B. pascuorum* is maintained in Sweden, which might be facilitated by a low overall genomic differentiation. We show that large-scale population genomic analyses of dry-pinned historical insect specimens are challenging but possible, and highlight their potential for improving our understanding of how anthropogenic global change is impacting insect populations.



High-throughput palaeogenomics reveals lost bovine diversity

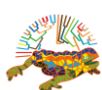
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Large-scale screening of DNA preservation in museum collections remains a limiting and costly factor in harnessing the potential of museomics. Robot-based solutions require trained technicians and costs can be prohibitive to museums. We introduce an alternative high-throughput DNA extraction protocol implemented on a 96-column plate. We validated the use of our method by comparing recovered screening metrics, such as endogenous DNA, to routine low-throughput single columns. Our plate method allows to generate 96 DNA extracts in approximatively 4 hours of laboratory

work, while bringing cost down by ~39%. As a case study, we applied our screening protocol to a collection of 200 bone fragments from Denisova Cave, Siberia. We retrieved mitogenomes that allowed us to assess past bovine diversity in Central Asia over a timespan of 200,000 years. Phylogenetic reconstructions even uncovered a previously unknown and extinct bovine lineage sister to all modern Himalayan yaks. Molecular dating suggests a split between both lineages as old as ~700,000 years ago.



Identification of a male Neanderthal from Prado Vargas, Spain, via enamel paleoproteomics

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Background: In recent years, the study of ancient protein remains (also known as paleoproteomics) has opened a window into the biology of extinct species and populations. Among its applications, the analysis of peptides derived from AMELX and AMELY genes—transcribed from the X and Y chromosomes, respectively—has proven especially valuable. These paralogs are expressed during enamel formation and can be used to determine the chromosomal sex of tooth remains. This study aims to analyse the protein content of a Neanderthal tooth from Prado Vargas, Spain, to infer the sex and corroborate the taxonomy assigned to this individual.

Materials and Methods: The sample was morphologically identified as a Neanderthal deciduous lower-left first molar (Ldm1); ancient DNA (aDNA) recovery was not possible due to clear evidence of resorbed roots. The tooth is dated to approximately 54.7–39.8 ka and likely belonged to a child who lost it ante mortem at around 9 to 10 years of age. Peptides were recovered from a 30 mg enamel fragment through an overnight acid dilution (10% TFA vol/vol) and a subsequent C18-based reversed-phase StageTip extraction. Protein identification

was performed using standardised liquid chromatography tandem mass spectrometry (LC-MS/MS) coupled with a database-dependent search via MaxQuant. Downstream data analysis was conducted using custom-developed scripts.

Results: After protein sequence reconstruction, we identified AMELY-specific peptides, denoting the individual as a male. We confidently identified amelogenin peptides featuring the 59-Met insertion, a sequence variant exclusive to AMELY and not present in AMELX. Phylogenetic analysis placed the individual within the hominin lineage and revealed enamelome Neanderthal-specific variants that need further investigation.

Conclusions: In the absence of aDNA, enamel paleoproteomics offered an alternative approach to determine the chromosomal sex of a Neanderthal individual. These findings highlight the potential of AMELX/AMELY-based sex determination to test sex-related hypotheses in archaeological and paleontological contexts, particularly in the case of immature individuals, whose morphology provides limited insight.



Museomic insights into temporal genomic change in the koala

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Background: The koala (*Phascolarctos cinereus*), an iconic marsupial endemic to Australia, faces significant conservation challenges due to habitat fragmentation, disease, and climate change. Despite extensive conservation efforts, current koala populations exhibit concerning signs of reduced genetic diversity. However, the extent to which this loss reflects long-term trends versus recent anthropogenic pressures remains unclear. Here, we use a museomic approach to assess changes in genomic diversity and structure across time and space in the koala, aiming to establish a baseline for future research and conservation management.

Materials and Methods: We generated and analysed whole-genome data from 37 historical koala specimens (dated from 1817 and 1980) sourced from museum collections, alongside 25 modern individuals representing current populations across koala range states. Ancient DNA was extracted in dedicated clean lab facilities, and sequencing libraries were prepared using the BEST-single tube protocol optimised for degraded DNA. Modern samples were sequenced using standard high-throughput methods. Population structure was investigated using principal component analysis and admixture modelling, while genomic diversity metrics - including heterozygosity and runs of homozygosity (ROH) - were estimated for both historical and modern datasets. Recent and past demographic histories were inferred through the investigation of linkage disequilibrium (LD) decay patterns (GONE) and the usage of Pairwise Sequentially Markovian Coalescent models (PSMC).

Results: Our analyses revealed a marked decline in genomic diversity over time, with modern koala populations showing significantly reduced

heterozygosity compared to historical individuals. This temporal comparison also revealed the disappearance of several mitochondrial haplotypes and nuclear genotypes, indicating the loss of unique genetic lineages over the past century. Population structure analyses uncovered a historically more diffuse genetic landscape, with some Queensland individuals interspersed among southern clusters, suggesting past gene flow across regions. In contrast, contemporary koalas show stronger genetic structuring and the absence of historically distinct Queensland genotypes—reflecting increased regional isolation, habitat fragmentation, and potential lineage loss. Linkage disequilibrium-based demographic reconstructions pointed to a sharp and recent decline in effective population size, consistent with known periods of intensive hunting and land-use change following European colonisation. Together, these results suggest that modern koalas are a genetically impoverished subset of a once more diverse and interconnected species.

Conclusions: This study demonstrates the power of ‘museomics’ to reveal historical baselines for species conservation. By comparing genomes from koalas past and present, we show that modern populations represent a genetically eroded subset of a once more diverse and interconnected species. These findings highlight the importance of considering historical diversity in conservation planning and underscore the need for strategies that promote genetic connectivity. More broadly, our results illustrate how museum specimens, when integrated with modern genomic tools, can provide crucial context for understanding the evolutionary and demographic trajectories of threatened species.



Museomic Reconstruction of Faunal Genomic History in the Hula Valley Since the Natufian

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At the heart of a key biogeographic corridor linking Africa, Asia, and Europe, the Hula Valley preserves one of the most archaeologically and ecologically informative wetland ecosystems in the Levant. Within this region lies the Eynan (Ain Mallaha) archaeological site—an exceptionally well-preserved Natufian settlement (~13,000 BP)—which offers critical insights into early human-animal interactions and faunal diversity on the cusp of agriculture. For paleontologists and evolutionary biologists, Eynan provides a rare temporal and environmental window into pre-domestication community structures and species distributions, offering stratified faunal material ideal for genomic analysis.

Our study integrates ancient, historical, and modern mammal samples—including the mountain gazelle (*Gazella gazella*), jungle cat (*Felis chaus*), and wildcat (*Felis silvestris*)—to explore how anthropogenic pressures and landscape modifications shaped genomic diversity and population structure across time. Many archaeological and museum specimens used in this research are

morphologically identified or partially classified; we assess whether degraded DNA can aid in species identification and resolve taxonomic ambiguity. We focus heavily on methodological comparisons, testing multiple aDNA extraction techniques and library preparation workflows—including in-house protocols and commercial kits such as NEBNext® Ultra™ II DNA Library Prep for Illumina—to identify the most effective approaches for working with humid-condition samples like those from the Hula Valley.

Through genome-wide sequencing and population-level analysis, we track evolutionary trends, genetic erosion, and ecological resilience from the Natufian period through the mid-20th century to the present. Our findings underscore the potential of museomics to recover lost diversity, refine historical baselines, and support future conservation. This project highlights the importance of integrating stratified archaeological material with natural history collections to build high-resolution evolutionary narratives in complex, human-modified ecosystems.



Temporal changes of genetic diversity in Finnish butterflies with varying population trends

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MEETING ROOM 120+121, August 21, 2025, 10:30 AM - 12:30 PM

Background: Insect declines have been reported worldwide, both in terms of species richness and population abundance. Land-use modification and climate change have been identified as key drivers of these declines. While species losses and population declines can be quantified through long-term biodiversity monitoring, losses in genetic diversity remain difficult to assess over time. Yet, genetic diversity underlies the adaptive potential of species and is therefore critical for their ability to respond to environmental changes. Long-term butterfly monitoring in Finland over the past 25 years indicates that species have responded differently to anthropogenic change, as reflected in varied population trends. This monitoring baseline, combined with a museomics approach, provides a unique opportunity to assess temporal dynamics of genetic diversity in species with different population trends and potentially to identify periods of significant genetic change.

Materials and methods: Using a museomics approach, we are investigating temporal changes in genetic diversity over the past century in five butterfly species exhibiting either stable or declining population trends. We sequenced whole genomes of museum specimens collected at three historical time points from two regions in southern Finland,

alongside their corresponding contemporary populations. After accounting for postmortem damage in the museum samples, we compared historical and contemporary populations of each species to detect signs of genetic erosion and increased inbreeding. To control for sequencing depth differences, we downsampled the coverage of contemporary populations to match that of the historical specimens and estimated genotype likelihoods.

Results: Preliminary findings indicate signs of a decline in genetic diversity from the 1980s to the present, with variation across species and regions. In one region, a species previously considered stable based on monitoring data also shows recent genetic decline. All species exhibited temporal genetic structuring, with contemporary populations being the most genetically distinct. However, differences in sequencing coverage between historical and contemporary samples complicate interpretation, raising the possibility that some signals may be technical artifacts rather than true biological patterns.

Conclusions: We will present our most recent findings and discuss the implications for understanding genetic erosion in relation to long-term population trends.



Temporal genomics reveals widespread but unexpected consequences of a bottleneck in the Scandinavian brown bear

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MEETING ROOM 120+121, August 20, 2025, 10:30 AM - 12:30 PM

Background: In recent years many species have gone through severe human-mediated population declines. Decreased population sizes make species vulnerable to the stochastic effects of genetic drift, and enhance the risk of inbreeding and inbreeding depression. Even when populations recover numerically, bottlenecks could have long-lasting consequences on population viability due to the loss of adaptive potential. The Scandinavian brown bear (*Ursus arctos*) was excessively hunted in the early 1900's, and by 1930 the population had decreased significantly. Eventually protective measures were taken and today population numbers have recovered. The population consists of two genetically distinct subpopulations that colonized Scandinavia from different directions; an eastern European lineage found in northern Scandinavia, and a western European lineage found in southern Scandinavia. Previous studies on the consequence of the bottleneck based on genetic markers and mitochondrial genomes have yielded contradictory results. While some studies argue that genetic diversity was unaffected by the bottleneck, others argue that the modern population has lost a lot of genetic variation, especially in southern Scandinavia. The aim of this study was to analyze whole-genome data from museum specimens and modern samples to further elucidate the consequence of the bottleneck in Scandinavian brown bears.

Materials and Methods: Data generation - We extracted DNA from 21 muscle tissue samples collected between 2007 and 2012, and from seven bone powder samples dating from 1832-1940. The samples were sequenced to a genomic coverage between 6X and 19X, but were later subsampled to 6X to avoid biases in downstream analyses.

Computational analyses: The GenErode v0.4.2 pipeline, or scripts adapted from it were used to map all samples and perform analyses of genomic erosion and population structure. We used Principal Component Analysis (PCA) to assess population structure of modern and historical

Scandinavian brown bears. For each sample, we estimated genome-wide heterozygosity using mlRho, inbreeding based on runs of homozygosity (ROH) and mutational load using SnpEff. In addition to this, we constructed a neighbor joining nuclear phylogeny of the 28 brown bears from this study and included published brown bear genomes from Finland and Hokkaido. We investigated gene flow by performing network analyses of the Y chromosome, as well as admixture graph modelling based on the whole genome data.

Results: Surprisingly, when comparing historical and modern samples, we found no significant temporal change in genome-wide heterozygosity, inbreeding levels or mutational load. Analyses of population structure, phylogeny and admixture clearly show that the subpopulations are more genetically similar now than prior to the bottleneck. The modern-south population shares more genetic similarities with modern-north than historical-south, and the results suggest that the contemporary population has lost the unique historical-south ancestry.

Conclusions: Despite the severeness of the bottleneck, the Scandinavian brown bear population shows no decrease in heterozygosity or sign of inbreeding depression. Furthermore, the post-bottleneck subpopulations are more genetically similar than the pre-bottleneck subpopulations. Together, these results suggest that gene flow, which can be density-dependent, increased after the bottleneck between the genetically distinct subpopulations and maintained heterozygosity in the population. These findings have implications for conservation actions, as they show that fragmentation of genetically structured populations may have less severe impacts than estimated. However, unique alleles can still be lost due to genetic drift, with possible consequences for the adaptive potential of populations.



The Museum Genomics of Bumblebees: Collections and Collecting for DNA.

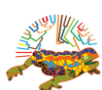
Barnes I¹

¹Natural History Museum

MEETING ROOM 120+121, August 21, 2025, 10:30 AM - 12:30 PM

Natural history museums have a unique and increasingly vital role in genome-focused research on non-model organisms. This role spans two key domains. First, museums serve as testbeds for the development of methods to recover genomic data from historical specimens, drawing on techniques pioneered in ancient DNA research. While museums have been described as “storehouses of DNA” since the early 1990s, recent advances in high-throughput sequencing and library preparation are now making it feasible to generate genomic data from degraded material at scale. Second, museums are central to the collection, curation, and storage of specimens for sequencing projects, particularly the development of reference genomes. Their cold-chain infrastructure and taxonomic expertise position them as an important element of biodiversity genomics programs. Here, I will show how we are leveraging museum collections to build genomic datasets that inform conservation efforts. Although vertebrates—especially humans and other mammals—have dominated

museomic research, our focus is increasingly shifting toward terrestrial insects, which comprise the majority of museum specimens. In particular, despite their prevalence in collections, economic importance, and important ongoing questions about their imminent decline, no work has been conducted on museum bumblebee collections to establish baselines of historical genome diversity. Our collections work for the Darwin Tree of Life project has provided high-quality reference genomes, in turn enabling us to develop the first large scale historical datasets for bumblebees. I will share findings from our studies using hundreds of specimens from British collections of two species, *Bombus muscorum* and *Bombus lapidarius*. While we find a broad reduction in diversity in *B. muscorum* over the twentieth century, the pattern for *B. lapidarius* is more complex. The work highlights both the technical challenges and opportunities of working with historical Hymenoptera specimens.



Uncovering Lost Diversity: Insights into the Genomic Structure and Population Dynamics of European Wolves using Museum Specimens

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MEETING ROOM 120+121, August 20, 2025, 10:30 AM - 12:30 PM

Background: Historically, grey wolves (*Canis lupus*) were widely distributed across Europe. Over the past two centuries, their numbers significantly declined due to habitat loss and human conflicts, leading to regional extinctions. Recent conservation and protection measures have facilitated increases in population numbers and recolonization of many areas. However, concerns remain about the impact of this recent population collapse on the ongoing health and viability of European wolves moving forward. Here we use historical museum and modern specimens to reconstruct the genomic makeup of European wolves before and after their population collapse.

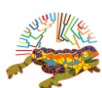
Materials and Methods: We sequenced the genomes of more than 1000 modern European wolves, which we combined with publicly available canid genomes to create a comprehensive dataset of 2500 individuals. In addition, we sequenced historical wolf specimens from museum collections, with a focus on Fennoscandia, using low-coverage whole-genome sequencing. To enable haplotype-based analysis we imputed 127 historical specimens with a coverage over 0.5X using our modern canid panel. This approach allowed us to track temporal shifts in genomic structure, assess genetic load, and examine introgression dynamics over time.

Results: Our analysis revealed strong geographic structuring among modern wolf populations, with clear regional differentiation. Historical Fennoscandinavian wolves exhibited a distinct genetic profile compared to modern wolves from the same area. Interestingly, this profile persisted in some captive individuals sampled in the

21st century, suggesting that captive populations may harbor genetic diversity that has been lost in the wild.

We also found that historical Fennoscandinavian wolves showed greater genetic affinities to ancient Holocene wolves, suggesting that population turnover in the 20th century led to the extinction of ancient haplotypes. Both historical and modern populations had a high proportion of their genome in runs of homozygosity. The high genetic load from before the population collapse highlights the fragile genetic health of wolves due to low population numbers. Despite recolonisation and increases in population numbers, wolves across many regions of Europe continue to carry high genetic load due to bottlenecks and founder effects. Notably, we find only limited dog introgression in historical or modern wolves, suggesting that reduced population sizes did not lead to widespread hybridization.

Conclusions: This study highlights the importance of using museum collections to reconstruct historical landscapes and temporal evolution in populations. By integrating genomes preserved in museum collections with modern data, we demonstrate how population bottlenecks and recolonization have shaped the current population dynamics of wolves across Europe. Our findings emphasize the importance of conserving both wild and captive lineages, particularly those preserving lost genetic variants and locally adapted haplotypes. Continued genetic monitoring – especially in populations with low diversity or from recent population foundations – will be essential to maintain the adaptive potential and health of European wolves moving forward.





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S38 - New Frontiers in Genome Diversity and Evolution: Exploring the 3D Organization and Function of Genomes



3D Genome Constrains Breakpoints of Inversions that Can Act as Barriers to Gene Flow in the Stickleback

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POSTER SESSION 2 - **P02.323**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

DNA within the nucleus is organized into a well-regulated three-dimensional (3D) structure. However, how such 3D genome structures influence speciation processes remains largely elusive. Recent studies have shown that 3D genome structures influence mutation rates, including the occurrence of chromosomal rearrangement. For example, breakpoints of chromosomal rearrangements tend to be located at topologically associating domain (TAD) boundaries. Here, we hypothesized that TAD structures may constrain the location of chromosomal inversions and thereby shape the genomic landscape of divergence between species with ongoing gene flow, given that inversions can act as barriers to gene flow. To test this hypothesis, we used a pair of Japanese stickleback species, *Gasterosteus nipponicus* (Japan Sea stickleback) and *G. aculeatus* (three-spined stickleback). We first constructed high-

quality genome assemblies of both species using high-fidelity long reads and high-resolution proximity ligation technologies and identified several chromosomal inversions. Second, via population genomic analyses, we revealed higher genetic differentiation in inverted regions than in colinear regions and no gene flow within inversions, which contrasts with the significant gene flow in colinear regions. Third, using Hi-C data, we revealed 3D genome structures of sticklebacks, such as A/B compartments and TADs. Finally, we found that inversion breakpoints tend to be located at TAD boundaries. Thus, our study demonstrates that the 3D genome constrains breakpoints of inversions that can act as barriers to gene flow in the stickleback. Further integration of 3D genome analyses with population genomics could provide novel insights into how the 3D genome influences speciation.



Ancestral origin and structural characteristics of non-syntenic homologous chromosomes in abalones

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POSTER SESSION 2 - P02.325, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Structural variation is increasingly recognized as a pivotal contributor to genomic diversity in marine invertebrates, yet its extent and evolutionary significance remain poorly characterized in many species. Haplotype-phased genome assembly is an excellent method for studying such variations by comparing homologous chromosomes. In this study, we constructed a haplotype-phased genome assembly for the western Pacific abalone, *Haliotis gigantea*, and subsequently analyzed its genomic features.

Materials and methods: High-fidelity (HiFi) long-read sequencing and high-resolution chromosome conformation capture (Hi-C) data were used for the genome assembly. The completeness of these genome assemblies was evaluated by complete single-copy Metazoa Benchmarking Universal Single-Copy Orthologs (BUSCO) genes. We examined the distribution of eight genomic features across scaffolds in the primary and alternative assemblies: counts of BUSCO genes, protein-coding genes, repeat elements, tandem duplication genes, segmental duplication genes, and structural variations (deletions, insertions, and

inversions). Comparisons were made between the two constructed assemblies and between them and the already published abalone genome assemblies.

Results: The primary and alternative assemblies each comprised 18 long scaffolds, consistent with the species' diploid chromosome number, and contained 96.5% and 96.2% Metazoa BUSCO genes, respectively, indicating high assembly quality. Comparative analysis of the two haplotypes revealed three homologous chromosomes with large-scale non-syntenic regions caused by extensive segmental duplications, each having fewer BUSCO genes and enriched in distinct gene domains. These non-syntenic homologous chromosomes likely originated in abalone evolution, as they were conserved across both closely and distantly related species.

Conclusion: Our findings highlight the evolutionary importance of non-syntenic structural variations in shaping genome architecture and suggest that such variations may play a broader role in functional diversification across abalones.



Comparative Genomics of Araneoidea Spiders reveal insights into their 90-million-year evolutionary history.

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¹University Of Kent

POSTER SESSION 2 - **P02.322**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

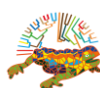
Uncovering the ancestral conservation of genomes, through ancestral reconstructions provide the novel opportunity to understand and quantify important chromosomal changes that might drive evolution and speciation events. Spiders (Araneae) are a diverse and ancient order, originating ~400 Million Years ago, and comprising of more than 52,000 species. Spiders are important both ecologically and economically, through their diverse adaptations and utilisation of venom and silk. Despite this Spiders have remained elusive within the comparative genomics field, largely hindered by the previously limited availability of sequenced genomes. However, with the influx of publicly available chromosomal-level assemblies, largely driven by sequencing initiatives, there is now the opportunity to explore the evolutionary genomic histories of Spiders.

We focused our analysis on Araneoidea of which are a diverse and widespread superfamily originating over 90 Million Years ago providing an ideal group to begin uncovering insights into the ancestral genome evolution of Spiders. We aimed to identify conserved ancestral gene groups and define ancestral chromosomal fragments across Araneoidea. We utilise 10 chromosomal level assemblies. BUSCOs were identified and used to initially construct a phylogeny of all spiders available at chromosomal level. BUSCOs were further utilised to infer ancestral gene groups using AGORA and Syngaph. Using DESCHRAMBLER we reconstructed predicted ancestral chromosomal fragments using whole-genome pairwise alignments.

Syngaph identified 12 ancestral linkage groups (ALGs) observed across all 10 analysed species. We identified a lineage-specific reciprocal translocation in *Argiope bruennichi* and *Gibbarenea gibbosa*, likely a shared characteristic of their shared ancestor. We observed further unique rearrangements across *G. gibbosa* not seen in the other species. *Trichonephila antipodiana* showed extensive rearrangement at chromosome ends, not shared in *T. clavata* and potentially indicative of a complex rearrangement history. AGORA identified contiguous ancestral regions (CARs) similarly present within Syngaph, supporting the identified ALGs.

We reconstructed predicted ancestral fragments using DESCHRAMBLER and determined the proportion of fragments present within each individual. We found similar levels of genomic conservation of the predicted ancestral genome, that were proportionate to the determined alignment rates. However, when placed fragments were mapped back onto the Araneoidea genomes, we saw extensive rearrangements of ancestral fragments, indicative of a complex genomic history.

Our analysis highlighted the complex evolutionary history underpinning Araneoidea, providing insight into their genomic structure, and how different tools highlight and uncover differing perspectives into the genomic characteristics underpinning their genomic evolution. These findings enhance our understanding of evolution in invertebrate genome structure and provide a foundation for further research within comparative spider genomics.



Divergent patterns of 3D genome organization across the Animal Tree of Life

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POSTER SESSION 2 - P02.326, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Understanding genome evolution is key to elucidating genome function. Within the Animal Tree of Life we can find very diverse sized genomes ranging from 19.7 Mb (*Pratylenchus coffeae*) to 130 Gb (*Protopterus aethiopicus*) genomes. This diversity is the result of genetic, ecological and evolutionary factors, which basically led to chromosomal reorganizations, including fusions, fissions, translocations and inversions. These CRs are critical for adaptation and speciation and can occur by virtue of genome plasticity. So far, studies examining the evolution of genomes have been mainly focused on sequence conservation. However, the inner working of a cell implies a tightly regulated crosstalk between complex gene networks, controlled by small dispersed regulatory elements of physically contacting DNA regions. How

these different levels of chromatin organization crosstalk in different species underpins the potential for genome evolutionary plasticity. Here we provide an overview on the evolution of chromatin organization, discussing on general aspects of the mode and tempo of genome evolution to later explore the multiple layers of genome organization. We propose that both genome and chromosome size modulate patterns of chromatin folding and that chromatin interactions facilitate the formation of lineage-specific chromosomal reorganizations. Overall, analyzing the mechanistic forces involved in the maintenance of chromatin structure and function of germ line is critical for understanding genome evolution, maintenance, and inheritance.



Dynamic reorganization and evolution of three-dimensional genome architecture during the species diversification of *Populus*

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¹Sichuan University

POSTER SESSION 2 - **P02.330**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Beyond the traditional linear perspective, rapid advancements in sequencing technology have enabled the exploration of genomic information in three-dimensional (3D) spatial structures. However, the genetic mechanisms underlying the evolution of higher-order chromatin structures and their role in driving species divergence and speciation remain largely unexplored. In this study, we constructed 3D genome architecture maps and conducted large-scale comparative analyses across eleven *Populus* species with diverse environmental adaptabilities and evolutionary relationships. Our aim is to investigate inter-species differences in 3D genome interactions within the genus *Populus* and to elucidate the relationship between 3D genome evolution and linear genomic changes, transcriptional regulation, and epigenetic modifications.

Method and Materials: In this study, we characterized the genetic features and evolution of 3D genome architecture by integrating super-pangenomes, RNA-seq, ATAC-seq, and Bisulfite-seq profiles across eleven *Populus* species that diverged along a speciation continuum. First, we investigated the genomic and epigenetic characteristics of A/B compartments and topologically associated domains (TADs) structures (including TAD regions and boundaries) that underlie 3D chromatin organization across species. Next, we assessed the evolutionary conservation of A/B compartments and TAD organization through comprehensive cross-species comparisons, analyzing how these features evolved along the divergence continuum and exploring the mechanisms driving the evolutionary dynamics of 3D genome structure. Finally, we examined the evolutionary connections between 3D chromatin reorganization, genomic variations, and epigenetic regulation, leveraging phylogenetic insights across the eleven *Populus* species along the divergence continuum.

Results: Our findings provide a comprehensive view of the distribution landscape of A/B

compartments, as well as TAD interiors and boundaries, across the genome both within and between species.

First, we found that the divergence of A/B compartments and TAD status increases with species divergence, with the evolutionary dynamics of TADs being significantly more active than A/B compartment switching between species.

Second, we observed that alterations in chromatin architecture across species are closely linked to genetic features, gene expression regulation, and epigenetic modifications. These changes are also subject to divergent evolutionary constraints when compared to conserved chromatin states.

Third, structural variations (SVs) along the linear genome were found to strongly correlate with changes in 3D chromatin architecture across species. A striking example of this divergence involves a 75-bp InDel in the 3' UTR region of the *HSFA2* gene, which differentiates species from phylogenetic clades I and II. This SV affects TAD boundary formation between the two clades and was verified to not only modify local chromatin looping between *HSFA2* and nearby genes but also impact the regulatory response to heat stress among species. These findings underscore the potentially critical role of SV-driven TAD reorganization in shaping novel regulatory landscapes, thereby influencing gene expression patterns and enabling species-specific adaptations to diverse environments.

Conclusion: By integrating comparative analyses of three-dimensional genome architecture with genomic and epigenetic features across eleven *Populus* species that diverged over tens of millions of years, our study offers valuable insights into the dynamics and evolutionary connections among 3D chromatin reorganization, genomic variations, and epigenetic regulation underlying genome divergence along the speciation continuum in non-model species such as forest trees.



Interspecies Functional Equivalence of Pancreatic Enhancers: Zebrafish as a Model to Study the Genetic Basis of Type 2 Diabetes

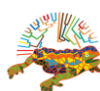
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POSTER SESSION 2 - **P02.329**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The endocrine pancreas plays a crucial role in regulating glucose homeostasis, and its dysfunction may lead to type 2 diabetes (T2D), a polygenic disease with rising global prevalence. Non-coding genetic variants associated with T2D have been identified through Genome-Wide Association Studies (GWAS). Most of these variants overlap with predictions of enhancers. This suggests a potential link between the malfunction of pancreatic enhancers and genetic susceptibility to T2D; however, in vivo models to study this hypothesis remain scarce. To improve zebrafish as an in vivo model, it is important to identify functional equivalent enhancers between human and zebrafish. Functional equivalent enhancers drive similar expression patterns across species. These may be orthologous enhancers, which share a common ancestral origin, or they may have evolved independently from different ancestral sequences, producing similar regulatory outputs via convergent evolution. When comparing zebrafish and human putative enhancers identified by chromatin profiles, only a minority is conserved at the sequence level. To address this limitation, functional equivalence between enhancers is inferred through a three-step approach: 1) identification of similar chromatin profiles within syntenic regions, 2) analysis of transcription factor binding sites (TFBS), and 3) enhancer reporter assays. Identifying functionally equivalent enhancers between zebrafish and human allows the study of how loss-of-function mutations in these regulatory sequences affect pancreas development and lead to a T2D phenotype. This work aimed to validate enhancers active in the zebrafish endocrine pancreas and identify their functional equivalents in the human genome. For this, the regulatory landscapes of 4 zebrafish genes (*foxa2*, *hnf1a*, *gck*, *neurod1*) were selected, as their human orthologs are important for β -cell function. Using ATAC-seq and H3K27ac ChIP-

seq data from the endocrine pancreatic islet of adult zebrafish (unpublished data from our lab), 23 putative pancreatic enhancers were identified within the regulatory landscapes of the 4 zebrafish genes. 18 putative enhancers were cloned into Z48 GFP reporter plasmids and injected into one-cell stage zebrafish embryos. The GFP expression in the endocrine pancreas was assessed in the F1 generation. 8 enhancers led to a strong and consistent GFP expression in the zebrafish endocrine pancreas, with 4 showing activity specifically in β -cells. The chromatin profiles of the gene regulatory landscapes from the 4 selected zebrafish genes were compared to those from the regulatory landscapes of their human orthologous genes. 4 human putative functional equivalent enhancers were identified, 1 for each of the 4 β -cell enhancers. A comparison of the TFBS of these 4 human enhancers to those from their putative zebrafish equivalents was performed. Significant numbers of common TFBS, particularly those important in endocrine pancreatic function, were observed in the 4 cases, supporting the hypothesis of functional equivalence. Using the LiftOver tool, 1 zebrafish enhancer of *foxa2* mapped to its human putative functional equivalent, covering 96.9% of the bases and 99.3% of the span of the original region, indicating a high degree of sequence conservation. This strongly supports the hypothesis that these two sequences can be functional equivalents and even orthologous enhancers. In the continuation of this project, the 4 human putative functional equivalent enhancers will be tested in zebrafish to assess if they drive expression patterns similar to those of their zebrafish counterparts. This work helped describe the zebrafish's endocrine pancreatic cis-regulatory landscape, thus improving this organism as an in vivo model to study the complex genetic component of T2D.



Long-term inversion recurrence and segmental duplication conservation in mammal genomes

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POSTER SESSION 2 - P02.332, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Segmental duplications (SDs) are highly identical duplicated DNA fragments longer than 1 kb, which contribute to recurring rearrangements through non-allelic homologous recombination (NAHR) and are a potential source of phenotypic diversity and disease. However, they are difficult to sequence and assemble reliably which makes them one of the least known regions of the genome. Recently, thanks to new long-read sequencing techniques and ongoing projects focused on building high-quality reference genomes, it is possible to investigate SD conservation and evolution across the mammalian phylogeny for the first time. In this study, we analysed 1164 human SD pairs throughout the genome, and assessed their evolution in 42 species with a wide range of divergence times. This allowed us to estimate the origin of each pair of SDs and observe differences in SD evolution depending on their orientation and type of chromosome. In general, 63.9% of the SDs have some level of conservation, although this proportion is higher for chromosome X (81.6% and 92.0% for direct and inverted

SDs, respectively). Moreover, chromosome X displays most differentiation in all SD characteristics related to the rate of recombination, such as the identity between the repeats. In inverted SDs, there is significantly higher identity in chromosome X compared to autosomes (P-value = 1.5×10^{-6}). However, for direct SDs we find a significantly lower identity in chromosome X (P-value = 4.7×10^{-3}), an opposite trend that can be related to their survival through the phylogeny. Interestingly, we identified 89 SDs (56 in direct and 33 inverted) that have their origin more than 80 mya, 31 of which (34.8%) are located in chromosome X. Lastly, for inverted SDs we found independent inversion recurrent events in different species for 49 of them, including several previously validated human NAHR inversions. Thus, these results help to identify the most conserved regions across mammal evolution and the main factors involved in this conservation, as a first step to determine their potential functional implications. In addition, they suggest that the genome is much more dynamic than previously thought.



On the genomic underpinnings of speciation in holocentric sedges

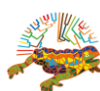
Venkatanarayana Mohan A¹

¹University Of Neuchatel

POSTER SESSION 2 - **P02.328**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The plant family Sedges (Cyperaceae) shows outstanding species diversity, coupled with high karyotypic diversity. Importantly, their chromosomes do not have a single centromere but are holocentric, i.e. with centromere-like regions across the entire chromosome length. Consequently, sedges, similar to other holocentric groups, are less likely to suffer from meiotic constraints associated with chromosomal fusions and fissions, showing high rates of karyotype evolution. While karyotype evolution has been suggested to promote reproductive isolation, whether and to what degree structural variants and chromosomal rearrangements that do not induce karyotype changes could promote speciation remains an open question. Here, sister species pairs with different and the same karyotypes offer a unique opportunity to study the potential association between chromosomal rearrangements, karyotype evolution, and speciation. In holocentric species, repetitive elements are hypothesized to be associated with chromosomal rearrangements and karyotype evolution. We established chromosome-level genome assemblies of three sister species pairs using somatic leaf tissue, long-read sequencing, and high-throughput chromosome conformation capture (Hi-C). We annotated the genomes for repeat elements and genes, measured the degree and number of rearrangements between each species pair, and identified genomic regions present at synteny breakpoints. In parallel, we obtained the 3D genome conformation in each species using Hi-C data and tested for the presence of

compartments and Topologically Associated Domains. Together with annotations, breakpoint region identity, and their 3D interaction matrices, we tested whether repetitive elements were enriched in breakpoint regions and tested for non-random associations of breakpoint regions with areas of higher contact frequencies in 3D, as these could result in areas of low recombination, resulting in reproductive isolation. We further obtained estimates of DNA methylation across these regions involved in genomic rearrangements to test for an association between DNA methylation levels (high or low) and syntenic breakpoint regions. Our results highlight that syntenic breakpoint regions often consist of different classes of repeat elements. In 3D, Carex genomes did not show strong compartmentalization, indicating that despite overall high levels of DNA methylation (50-60%), sedge genomes in somatic tissue do not organize into eu- and heterochromatin regions. TADs are mostly absent in sedge genomes, with a few TAD-like regions showing higher frequencies of contact, albeit with low insulation scores, which may indicate ancestral linkage groups that have undergone recent fusion events. Our study brings together a suite of investigations to understand the role of structural variants, genomic rearrangements, and karyotypic changes in the evolution of reproductive isolation and speciation in holocentric species, which account for 20% of all known extant plant and animal taxa on Earth.



Patterns of Genome Reshuffling and 3D Chromatin Architecture in Turtles

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POSTER SESSION 2 - P02.331, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Three-dimensional (3D) genome architecture is essential for understanding gene regulation, genome function and evolutionary plasticity. Chromatin interactions between distant genomic loci are non-random and species-specific, contributing to the structural and functional complexity of the nucleus. However, data on 3D genome organization in non-model vertebrates remain scarce. To explore the macroevolutionary diversity of chromatin conformation in vertebrates, we performed a comparative analysis of three cryptodiran (hidden-neck) turtle species representing different evolutionary lineages and sex determination systems: *Apalone spinifera* (ZZ/ZW, 2n = 66), *Staurotypus triporcatus* (XX/XY, 2n = 54), and *Testudo hermanni* (temperature-dependent sex determination, 2n = 52). By generating de novo genome assemblies using Illumina sequencing and chromosome conformation capture, we uncovered key differences in 3D chromatin organization among these species. We detected differences in 3D chromatin structure in turtles compared to other amniotes beyond the fusion/fission events detected in linear genomes. Our results reveal different degrees of genome rearrangement. *Apalone* (Trionychidae) maintains a highly conserved karyotype, suggesting genomic stasis and

similarity to the inferred ancestral amniote state. In contrast, *Staurotypus* (Kinosternidae) and *Trachemys scripta* (Emydidae) show moderate levels of chromosomal fusions and fissions, reflecting a more dynamic karyotypic evolution. These structural variations are accompanied by distinct patterns of chromatin folding. We identified a novel chromatin organization pattern involving 'centromere-telomere interactions' in all three turtles, an arrangement not previously detected in turtles. Together with 'centromere clustering' (a feature known in sauropsids), this configuration constitutes a unique 3D nuclear architecture among amniotes, challenging previous models of chromatin topology in vertebrate evolution. Our hypothesis is that the divergent pattern found in turtles originated from an amniote ancestral state defined by a nuclear configuration with extensive associations between micro-chromosomes, which was preserved during the reshuffling of the linear genome. These findings provide new insights into the evolution of vertebrate genome organization, highlight the influence of karyotypic diversity and sex determination systems on nuclear architecture, and underscore the value of integrating 3D genomics in non-model species to reveal conserved and lineage-specific patterns of chromatin evolution.



The structure of mitochondrial genomes across the plant tree of life

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POSTER SESSION 2 - **P02.324**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Plant mitochondrial genomes are structurally dynamic, showing remarkable variation in size, gene content, and recombination patterns across lineages. Despite their importance in metabolism, development, and cytoplasmic inheritance, the evolutionary landscape of mitochondrial genome structure across plants remains poorly understood—largely due to assembly challenges posed by repeats, multipartite architectures, and structural isomers [1, 2]. We aim to characterise the diversity and structure of plant mitochondrial genomes through a DNA sequence graph-based lens. Leveraging over 200 mitochondrial genome assemblies from the Darwin Tree of Life project [3], spanning the diversity of vascular and non-vascular plants, we examine how genome architecture evolves in a phylogenetic context and assess lineage-specific patterns of gene content, repeat structure, and putative transposable element activity.

Our approach centres on the analysis of assembly graphs from the new “oatk” assembler, which preserve structural complexity often lost in linearised genomes [4]. These graphs enable us to detect alternative genome configurations, infer recombination-prone regions, and map the physical context of mitochondrial genes and repeats. By comparing graph structures across lineages, we identify trends in repeat proliferation and recombination frequency that reflect deeper evolutionary processes. Preliminary findings suggest that genome structure is not randomly variable but instead shows phylogenetically patterned

constraints, with certain clades exhibiting stable architectures and others showing signs of frequent recombination. Gene content shows variation across clades and offers insights into potential nuclear integration events. In several cases, we also observe signals suggestive of transposable elements, raising questions about the role of mobile DNA in shaping mitochondrial genome evolution—either directly or via nuclear-mediated integration.

Our work provides a comparative framework for understanding plant mitochondrial genome evolution that embraces structural complexity. By analysing genomes in their native graph form, we open new avenues for exploring recombination, constraint, and innovation in plant mitochondrial genomes.

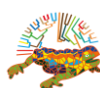
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Understanding extreme heterochiasmy: cellular and genomic insights in frog systems

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POSTER SESSION 2 - **P02.327**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

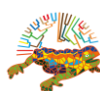
Meiotic recombination is a major driver of diversity across the Tree of Life. The recombination landscape differs strikingly between sexes, a phenomenon known as heterochiasmy, with females typically exhibiting higher recombination rates than males. In addition, recombination suppression between the sex chromosome pair is the hallmark signature of sex chromosome evolution. In this context, our research aims to investigate the evolutionary forces and genetic basis of sexual dimorphism in recombination landscape, and to assess its impact on sex chromosome evolution which remain poorly understood.

Frogs are excellent organisms to investigate the relationship between sexually dimorphic recombination landscape and sex chromosome evolution as they possess early-stage, homomorphic sex chromosomes, and have many lineages with extreme heterochiasmy, such as genome-wide, male-specific telomere-restricted recombination. Many species across diverse frog lineages majority with XX/XY, a few with ZW/ZZ sex chromosome systems have extreme male-specific recombination suppression, with females displaying more uniform pattern, suggesting it might be an ancestral trait, pending further investigation. In line with this, we hypothesize that extreme heterochiasmy in frogs is governed by sex-specific meiotic regulation, possibly including transcriptional regulation, epigenetic mechanisms, and/or chromatin modifications. To uncover the cellular and genomic basis of this pattern, we combine cytogenetic and transcriptomic approaches in the European common frog (*Rana temporaria*) which exhibits homomorphic XX/XY sex chromosome system and persistent extreme heterochiasmy.

As the initial step to study the recombination landscape along with the underlying suppression mechanisms, we assessed the timing of meiosis I in this species and captured meiotic prophase I cells. Immunostaining of juvenile female gonads showed clear signal of prophase I, characterized by pronounced γ H2AX signals at its start and high RNA polymerase activity in diplotene-arrested oocytes,

confirming successful capture of female meiosis I and transcriptionally active lampbrush chromosomes in *Rana temporaria* for the first time. Secondly, we investigated the role of meiotic prophase I related genes in sex-specific recombination regulation by analyzing their differential expression between female and male frogs. Using RNA-seq data of both developmental and adult tissues, from four populations across Switzerland, Sweden, and Finland, with extreme heterogeneity in Y chromosome differentiation levels, we identified sex-biased and sex-specific gene expression patterns. This allowed us to link prophase I gene expression to population-specific levels of sex chromosome differentiation. Recombination suppression could be driven by epigenetic modifications that alter chromatin structure, limiting access to recombination machinery or silencing chromatid or chromosome pairing elements in a sex-specific manner. Given the temporal differences in meiosis between sexes, meiosis-related genes are expected to be testis-biased in adults and ovary-biased in early developmental stages. Our hypothesis predicts either unbiased expression or reduced expression of certain genes involved in synapsis and crossover formation in adult testis tissue, attributed to extreme heterochiasmy. Consistently, expression of several genes involved in homologous chromosome pairing and crossover resolution such as *Sycp1* and *Mlh1* were female-biased in late developmental stages. Furthermore, we identified significant sex-specific expression differences in meiotic cohesin complex (testis-specific *Rec8*-type), synaptonemal complex (testis-specific *Sycp3*), and recombinase complex (male-biased *Dmc1* and male-specific *Brme1* recombinase recruitment). Next, we will employ immunofluorescent cytology to label candidate proteins alongside crossover sites, coupled with epigenetic staining in both sexes.

This work lays the foundation for understanding the genetic and molecular mechanisms driving sex-specific regulation of recombination in Anurans which has evolutionary implications for sex chromosome evolution across all vertebrates.



Unraveling the Phenotypic Evolution and Global Breeding History of Domestic Chickens

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POSTER SESSION 2 - P02.335, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

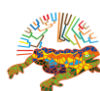
Background: Chickens are the most ubiquitous and genetically diverse domestic animal on Earth, deeply embedded in human culture, subsistence, and scientific discovery. Despite their biological and societal importance, the evolutionary history and genetic architecture underlying their global phenotypic diversity remain poorly resolved. Here, we leverage the most comprehensive chicken genomic dataset to date—comprising 11,088 whole-genome sequences from 394 domestic breeds across all continents, alongside 1,071 genomes from four species of wild junglefowls—to investigate the origins, dispersal, and phenotypic evolution of domestic chickens.

Results:

Our integrative analyses reconstruct the global breeding history of chickens and reveal how complex traits such as body size,

skin pigmentation, and feather morphology evolved through a mosaic of ancient introgression, parallel selection, and breed-specific adaptation. We uncover distinct genetic signatures linked to domestication, adaptation, and trait convergence across isolated lineages. Moreover, our findings highlight how the spread of chickens has paralleled major waves of human migration and agricultural expansion since the Middle Holocene.

This study not only illuminates the evolutionary processes shaping domestic chicken diversity but also offers a genomic framework for future sustainable breeding. Our results provide critical insights into domestication, the genetic basis of adaptation, and the co-evolutionary narrative between humans and their most widely distributed avian companion.



An in-vivo approach to population 3D genomics: age and sex affect 3D genome in a healthy controlled cohort of chickens

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MEETING ROOM 118+119, August 19, 2025, 11:00 AM - 1:00 PM

While the variation in linear DNA sequence at the population level has been extensively studied, the three-dimensional (3D) folding of the genome and its diversity across individuals within healthy populations remains largely unexplored. This gap persists despite the growing interest in 3D genome organization and its regulatory mechanisms over the last decade. Major obstacles include the need for well-controlled cohorts, mostly having to be raised ad-hoc for this purpose, and the lack of cost-effective, replicable methodologies for generating Hi-C data.

This study addresses this knowledge gap by investigating how 3D genomic structure varies in a cohort of fast-growth broiler chickens (*Gallus gallus*) raised under closely controlled conditions. We generated Hi-C data from liver samples of 16 chickens (8 males and 8 females; with one replicate per sample, total $n = 32$), with balanced sampling at two life stages (day 7 and day 35). For each sample, we also collected RNA profiles and DNA sequence data. We analyzed and quantified 3D genome structure differences at multiple levels, from interchromosomal contacts to looping, compartmentalization, and topologically associating domains (TADs).

Principal component analysis (PCA) using ~4 million autosomal SNPs showed no

clustering patterns for our individuals. However, Hi-C data revealed a stark clustering by age and sex with large proportions of variance explained, particularly notable at the level of genomic compartments. Other large-scale 3D genome features, such as interchromosomal contacts, long-range compartmentalization and long-range decay contact functions also showed sex-specific trajectories between days 7 and 35.

Integration of Hi-C with RNA data highlighted several sex-specific differentially expressed genes important for liver metabolism. Additionally, from a dataset of chromatin structure remodeling genes, we identified several sex-specific differentially expressed genes, potentially accounting for sex-specific differences seen in 3D structure.

To our knowledge, this is the first attempt to characterize population-level variation in 3D genome structure across sex and life stages in a healthy animal population. Our results highlight how often-overlooked variables such as age and sex condition 3D genome organization in a tissue-specific manner. This proof-of-concept study provides a reference framework for future analyses of population-level 3D genomic variation and raises considerations for interpreting 3D structural features without accounting for common population variation.



Chromosome-level genomics and historical museum collections reveal new insights into the evolution of waterbuck

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MEETING ROOM 118+119, August 19, 2025, 11:00 AM - 1:00 PM

Background: Advances in the sequencing and assembly of chromosome-level genome assemblies have enabled the study of non-model animals, providing new insights into the evolution of genomes and chromosomes. Here, we present the waterbuck (*Kobus ellipsiprymnus*) as an emerging model antelope for studying population dynamics and chromosome evolution. Antelope evolutionary history has been shaped by Robertsonian (Rb) fusions, with waterbuck also showing variation in karyotype due to two polymorphic Rb fusions. These polymorphisms are variable between and within the two recognised subspecies, the common and defassa waterbuck.

Material and methods: To provide new insights into waterbuck evolution, we generated a chromosome-level genome assembly for the defassa waterbuck using PacBio HiFi and Hi-C sequencing. We further leveraged museum collections to perform whole genome sequencing (WGS) of 24 historical specimens from both subspecies.

Combined with a previous WGS dataset (n = 119), this represents the largest study of waterbuck populations to date.

Results: Our analyses reveal previously unrecognized population structure and gene flow between waterbuck populations, alongside regions with high genomic differentiation between the two subspecies. Notably, several differentiation hotspots occur near the centromeres of fixed and polymorphic Rb fusions, exhibiting signatures of low recombination and local population structure. These regions contain genes involved in development, fertility, and recombination.

Conclusions: Our findings underscore the value of chromosome-level genome assemblies, the critical role of historical collections in capturing fine-scale population structure, and the potential evolutionary impacts of Rb fusions on genomic differentiation and recombination landscapes.



Comparative Genomics to Unravel Chromosomal Instability in Butterflies

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MEETING ROOM 118+119, August 19, 2025, 11:00 AM - 1:00 PM

Large-scale chromosomal rearrangements may act as reproductive barriers, contributing to the formation of new species. Lepidoptera, comprising butterflies and moths, is among the most diverse taxonomic groups across the Tree of Life. All Lepidoptera have holocentric chromosomes without defined centromeres and therefore, chromosomal fusions and fissions may be less deleterious and more likely to be retained. Accordingly, some butterfly clades show a tremendous diversity in chromosome numbers. These clades often show bursts of species diversity, suggesting a role of rearrangements in species diversification. To understand why some clades show such a high rate of chromosomal fusions and fissions, while others have conserved chromosomes, we generated and compared chromosome-scale genome assemblies for

37 *Erebia* butterfly species. We ask if differences in rates of chromosomal rearrangements, associated with different rates of speciation between chromosomally stable and unstable lineages, are explained by (i) differential repetitive element expansions, (ii) differences in gene gains and losses, including DNA repair genes, and/or (iii) differences in 3D genome structure. In particular, we find that fusions and fissions of chromosomes are more likely at boundaries between 3D genome features, and that some of these 3D features are deeply evolutionarily conserved despite extensive chromosomal rearrangements. Overall, we disentangle the association between genomic features, chromosomal rearrangements, and species diversification for a highly diverse group with holocentric chromosomes.



Evolution of 3D chromatin architecture of brown algal genomes

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MEETING ROOM 118+119, August 19, 2025, 11:00 AM - 1:00 PM

Eukaryotic genomes encode genetic information in their linear sequence, but appropriate expression of their genes requires chromosomes to fold into complex three-dimensional (3D) structures. Whilst the distribution of heterochromatin and euchromatin can influence gene expression and contribute to phenotypic variation between species, how 3D genome architecture evolves within major lineages, how it changes over evolutionary time, and how these changes correlate with life history traits remain poorly understood. Here, we provide a comprehensive comparison of 3D chromatin architecture across six species of brown algae and one outgroup, representing the phylogenetic diversity, sexual systems and morphological complexity of the lineage. We investigate chromatin folding patterns and gene expression in autosomes and sex chromosomes, and we computationally

reconstruct ancestral karyotypes and syntenic relationships along the brown algal phylogeny, revealing the linear rearrangement patterns of brown algal chromosomes and linking these changes with the 3D nuclear architecture over approximately 400 million years of evolution. Finally, we unveil the structure and dynamic evolution of brown algal centromeres. Identification of the centromeric regions revealed long-term co-evolution with a specific lineage of “centrophilic” LTR retrotransposons, which are uniquely present at the centromeres in most species and likely constitute the centromeric repeat. The centromeres are also hotspots for chromosomal rearrangements; we characterize several Robertsonian translocations and centric insertions that have occurred in brown algal evolution.



New insights into chromatin organization and gene regulation in the germ line of vertebrates

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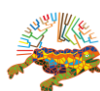
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MEETING ROOM 118+119, August 19, 2025, 11:00 AM - 1:00 PM

The study of the evolutionary plasticity and function of the higher-order vertebrate genome organization, and how this is transmitted to the offspring is fundamental for deciphering the mechanism(s) responsible for the origin and plasticity of genomes. Yet, our understanding of the mechanisms regulating genome folding and gene expression during the generation of germ cells in divergent vertebrate species is incomplete. By combining cytological analysis, fluorescence activated cell sorting, in situ chromosome conformation capture sequencing (Hi-C) and single cell-RNAseq, we describe principles of 3D genome folding and expression in the germ line of different vertebrate species (eutherian, marsupials and reptiles). Our results show unique patterns on the diversity of the spatial folding

of chromosomes in meiotic (primary spermatocytes) and post-meiotic cells (round spermatids). Importantly, our results show that both chromosome morphology and genome size strongly influence DNA loop size and inter-chromosomal interactions in meiocytes. Moreover, we explored the crosstalk between 3D genome remodelling and gene expression in the context of the meiotic sex chromosome inactivation (MSCI). We found that certain genomic regions in the tammar wallaby were permeable to MSCI, suggesting that silencing is not complete in marsupials. Overall, our results provide new insights into the regulation of chromatin in the germ line of vertebrates at an unprecedented level of resolution, with implications for genome organization and evolution.



The combination of population-level PacBio HiFi and transcriptome data enables the investigation of structural variants in regulatory evolution and speciation of two songbirds

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MEETING ROOM 118+119, August 19, 2025, 11:00 AM - 1:00 PM

The recent rise of long-read sequencing technologies has significantly improved our ability to identify structural variants (SVs), typically defined as mutations affecting more than 50bp. As a result, increasing empirical evidence demonstrates that SVs encompass a significant portion of the genome and can have large phenotypic effects. In addition, structural variants may play a substantial role in speciation, which could previously have been overlooked because of difficulties in identifying them with short-read data. However, due to the cost of long-read sequencing data, SV detection between species is still frequently based on the alignment and comparison of reference genome assemblies rather than population-level data, which does not permit distinguishing between polymorphic SVs and those that are differentially fixed between species. To address this limitation, we generate population-level long-read sequencing data of 10 collared flycatchers (*Ficedula albicollis*) and 10 pied flycatcher (*Ficedula hypoleuca*) individuals, an ecological model system of speciation research with approximately 6% mixed

breeding pairs and fully sterile F1 hybrids. This experimental setting enables us to show that only a small fraction (<1%) of SVs identified by alignment of the two reference genome assemblies are differently fixed between the two species. The vast majority of those differentially fixed SVs is represented by INDELs, with only a handful fixed inversions and translocations. We then use this dataset to study the association of SVs on regulatory mechanisms, separately for SVs segregating in one or both species and differentially fixed SVs. This analysis reveals that, polymorphic inversions and translocations are associated with a local reduction of nucleotide diversity within species and elevated levels of differentiation between species, consistent with local suppression of recombination and a mechanistic role of SVs in speciation. Ongoing work also assesses the role of SVs in gene regulatory evolution between species, where generation of transcriptome data for the same 20 individuals provides a powerful setting to identify cis-regulatory variants.





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**S39 - Novel experimental and
computational approaches to understand
the prevalence of reticulate evolution in
eukaryotes**



Beyond vertical inheritance: the dynamic evolution of transposable elements in *Drosophila*

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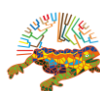
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POSTER SESSION 2 - P02.337, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

We synthesize recent genomic investigations across hundreds of *Drosophila* species and populations to document the scale, geography, and tempo of horizontal transposon transfers (HTTs). We show that 12 transposable elements (TEs) have invaded the *Drosophila melanogaster* genome over the last two centuries via HTT, and we trace the evolutionary origins of all TE families in the species, revealing Afrotropical and Neotropical *Drosophila* species as major exchange partners. One DNA transposon, Transib1, swept through all *D. melanogaster* populations worldwide within just two years of its initial appearance. Strikingly, two LTR retrotransposons, Shellder and Spink, have independently

invaded multiple *Drosophila* species within the past 50 years, spreading across continents and reaching even isolated island endemics.

These rapid, cascading transfers provide rare empirical evidence that HTT can occur and propagate on remarkably short timescales. By documenting these events in near real time and at high phylogenetic and geographic resolution, our findings not only underscore HTT as an active and ongoing force in eukaryotic genome evolution, but also represent one of the clearest examples to date of reticulate evolution unfolding over contemporary evolutionary timescales.



Hybrid Speciation? A Case Study in Grasshopper Species

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POSTER SESSION 2 - **P02.338**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Long dismissed as a rarity, hybridization is now seen as a pervasive and powerful force in evolution. It has the potential to “reverse” speciation, homogenizing previously differentiated taxa but it can also drive speciation by producing novel phenotypes and immediate reproductive barriers. While hybrid speciation has been well documented in plants, evidence in animals remains limited, and often suggestive based on phenotype alone. The “omics” era has provided new tools to test scenarios of hybrid speciation, which require the new lineage (1) to be phenotypically intermediate, (2) genetically distinct, (3) reproductive isolated from both parental species, and (4) present a mosaic genome landscape. The grasshopper *Chorthippus jutlandica* likely evolved within the last 12,000 years and has been hypothesized to originate from hybridization between *C. biguttulus* and *C. brunneus*, based on male song similarities between F1 hybrids and *C. jutlandica* individuals. This makes *C. jutlandica* an ideal system to study how gene flow contributes to the rapid evolution of behavioral isolation via sexual selection. Using forewing morphometric analyses, we demonstrate that *C. jutlandica* is

phenotypically intermediate between the two putative parental species. Furthermore, genomic markers show that *C. jutlandica* is a genetically distinct lineage, more similar to its parapatric neighbor *C. brunneus* than to the geographically isolated *C. biguttulus*. However, phylogenetic analyses place *C. jutlandica* as sister to *C. biguttulus*, suggesting that it diverged from *C. biguttulus* while receiving substantial gene flow from the parapatric *C. brunneus* —a pattern consistent with hybrid speciation. In addition, supporting a scenario of hybrid speciation, previous behavioral studies showed the reproductive isolation of *C. jutlandica* from the parental *C. brunneus*. Ongoing demographic and introgression analyses will clarify the timing and direction of gene flow and further test the hypothesis of reticulate evolution of *C. jutlandica*. Taken together, our findings already highlight *C. jutlandica* as a distinct evolutionary lineage shaped by divergence with potential gene flow. Our work will further disentangle how behavioral isolation can drive rapid speciation and the potential role of hybridization in this process.



Phylogeographic signatures of lineage fusion: a simulation study assessing the incorporation of geo-spatial information, and long-read nuclear haplotype data

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POSTER SESSION 2 - P02.339, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Secondary contact between long isolated populations can result in complete merging, such that parental lineages are no longer extant (“lineage fusion” [LF]). This scenario has rarely been explicitly considered in single-species and comparative phylogeographic studies, yet it has the potential to impact inferences about demographic history, and levels of congruence among co-distributed species. Given a sufficient number of loci and the use of efficient summary statistics, insights into whether a contemporary population gene pool has a history of past LF are attainable. However, there are several issues. First, as the ratio between pre-fusion divergence time vs. post-fusion gene pool sampling lag time (measured in N_e generations) becomes smaller, accurate inference becomes more challenging. Thus, it can be difficult to detect isolation-reconnection occurring over relatively short timescales. Second, asymmetric genetic contributions from each

of the two archaic parental lineages can dampen signatures of LF. Accordingly, LF events may be less readily detectable in landscape settings that contained several differently- (cf. uniformly-) sized Pleistocene refugia. Third, certain non-fusion demographic events produce similar hallmarks as LF -- particularly population decline. This means that in the absence of versatile methods that can be used to reliably infer LF, population decline may be erroneously inferred, simply due to not considering LF in the scenario set. Two potentially powerful solutions to these issues exist: (1) the incorporation of geo-spatial information, and (2) the advent of long-read (>10-kb) sequencing for generating multi-locus nuclear haplotype data. Here, I will report on outcomes of spatially explicit simulations of LF scenarios using a range of divergence : lag time ratios, and a range of genetic dataset compositions (number of sampled alleles, haplotype length, and number of loci).



Genome polarisation for detecting and characterising admixture

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MEETING ROOM 131, August 21, 2025, 3:30 PM - 5:00 PM

Admixture and reticulate evolution are under reported because of both denialism and a lack of appropriate inference tools. Genome polarisation uses the linkage disequilibrium generated by admixture to colour a set of genomes by their sources. The algorithm uses no taxonomic priors and scales in linear time to large genomes. Data can be resequencing, reduced representation, or mixed. The approach is unbiased by missing data. Applications are diverse. For a hybrid

zone we have shown genome regions identified as barriers to geneflow by polarisation correspond to regions shown to have reduced effective migration using the coalescent. We summarise further results for conservation and invasion biology, and adaptive introgression, discuss how the approach can inform us in general terms about which genome regions, and which parts of the 'tree' of life, are susceptible to admixture.



Reticulate evolution in a plant radiation: the role of introgression in shaping ecological diversity in the subgenus *Tillandsia*

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MEETING ROOM 131, August 21, 2025, 3:30 PM - 5:00 PM

Evolutionary radiations have contributed substantially to Earth's biodiversity, yet the precise mechanisms driving rapid diversification remain elusive. Recent research has revealed that hybridization between radiating lineages can result in adaptive introgression and may enhance rapid diversification. The bromeliad subgenus *Tillandsia* represents an exemplary case of evolutionary radiation, exhibiting multiple key innovation traits linked to elevated diversification rates, including CAM photosynthesis, tank formation, distinct pollination syndromes and growth on different substrates. The subgenus is thought to have arisen 7 mya in the Andes, where a South American lineage remains, and dispersed towards Central and North America 3-4 mya, where it diversified into a species rich set of lineages. In this study, we make use of a targeted sequencing approach with a Bromeliad-specific bait set of over 1,700 genes including hundreds of known genes involved in key innovation traits to investigate the evolutionary history of and prevalence of reticulate evolution in 35 *Tillandsia* species encompassing the ecological diversity of the radiation. We

found pervasive gene flow across the subgenus, yet gene flow appears more common between species that share certain key innovation traits such as the same growth habit, photosynthetic metabolism or density of absorptive trichomes. By investigating signatures of gene flow at synonymous and non-synonymous sites across targeted genes, we found that, while introgression is more often maintained at synonymous sites, more gene flow is detected at non-synonymous sites within key innovation genes than in other genes. McDonald-Kreitman tests across 13 species revealed 36 genes related to key innovation traits putatively under selection, with substantial sharing of candidate genes among species with similar traits. Our findings suggest that key innovation traits are generally associated with the maintenance of species barriers, but that introgression among species with similar ecology may have been less deleterious at genes associated with key innovation traits than in other genes. Our results point at a potential widespread role of introgression in shaping and maintaining ecological diversity in the subgenus *Tillandsia*.





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S40 - Phylogenomics methodology and the deep tree of life



Co-evolution of the Myosin Vb–Rab11 Interface and Villification of the Gnathostome Intestine

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¹Umcg

POSTER SESSION 3 - **P03.172**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Rab11-positive endosomes orchestrate intestinal mucosal development across a wide range of species by regulating apical membrane trafficking and epithelial polarity. Loss of the Rab11-binding partner myosin Vb disrupts this process in jawed vertebrates like humans, mice, and zebrafish, leading to severe villus atrophy and impaired nutrient absorption. Intriguingly, invertebrates like roundworms show no such defects upon myosin V deletion, suggesting evolutionary divergence in endosomal trafficking control and epithelial morphogenesis.

Materials and Methods: We performed comparative genomic analyses to trace the evolution of myosin V(b)'s cargo-binding domain (CBD), which mediates Rab11 interactions. Functional binding assays were conducted in vertebrate cell models to test myosin V CBD–Rab11 interactions. Additionally, we compared intestinal morphology across representative species using histological and imaging data to explore structural correlates of molecular divergence.

Results: The osteichthyes, bony fish including tetrapods (e.g., mammals) and

teleosts (e.g., zebrafish), uniquely retained an ancient fungal-derived Rab11-binding interface in their CBD, critical for directing polarized endosomal traffic. In contrast, invertebrates and vertebrate chondrichthyans (cartilaginous fish, including sharks) lost this interface over evolutionary time. Functional tests confirmed the divergence: chondrichthyan and invertebrate myosin V CBDs failed to bind Rab11 or disrupt endosome dynamics in vertebrate cells, unlike their tetrapod/teleost counterparts. Morphologically, this split aligned with intestinal wall morphology: tetrapods and teleosts evolved villi to maximize nutrient absorption, while invertebrates and chondrichthyans did not.

Conclusions: Our findings suggest that the myosin V–Rab11 interface co-evolved with villus development in jawed vertebrates, enabling precise control of endosome trafficking to allow the development of complex mucosal folds. This molecular adaptation likely supported metabolic demands as vertebrates diversified, highlighting how conserved trafficking machinery can be repurposed to drive evolutionary innovation in organ structure.



DBuilder: Automated Building of Proteome Database for Phylome Reconstruction

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POSTER SESSION 3 - P03.172, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Phylomizer is a pipeline designed to search for homologs, build alignments, and reconstruct the phylogeny of all proteins of a species of interest (seed) with a customized phylogenetic background. Downloading and formatting the mandatory proteome database for Phylomizer can be a complex and laborious task, as it requires integrating proteomes from different sources, selecting one protein per gene, filtering isoforms by length, adjusting headers, and concatenating proteomes. To address this database assembly, we developed DBuilder. We used the Python 3.7 programming language, following the object-oriented programming paradigm, with the VSCode source code editor. Code development involved a variety of libraries, including system and file management (sys, os, shutil, subprocess, gzip), user interaction and GUI - graphical user interface- (argparse, tkinter), data processing and manipulation (io, re), bioinformatics tools (Bio), web requests (Requests), image handling (PIL), logging (logging), and threading (threading). Finally, we documented and packaged the project using Sphinx and Setuptools, respectively.

DBuilder is modularized as follows:

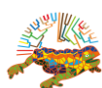
Two core modules, UniProt_batch_downloader.py and NCBI_batch_downloader.py, which download, uncompress, and rename proteomes. Optionally, they concatenate the downloaded proteomes and modify the sequence headers to match Phylomizer. NCBI_batch_downloader.py includes

additional functions to remove mitochondrial sequences and to preserve the longest isoform for proteins with multiple isoforms.

A main script, DBuilder_main.py, which executes the core modules by directly receiving input arguments instead of managing them through a command-line parsing mechanism.

Two secondary modules, DBuilder_GUI.py, which provides the ability to launch a GUI from the main script, and decorators.py, which contains the decorator used for logging.

DBuilder proposes a solution to the challenge of building databases for Phylomizer through a programmatic approach supported by Python and its powerful ecosystem of libraries. Its versatile nature offers an intuitive graphical interface for those with no programming experience and open source for those who want to customize the tool to their own specific needs. As Phylomizer constitutes the core engine behind PhylomeDB -the repository dedicated to genome-wide collections of gene phylogenies- future developments could integrate DBuilder into this platform. Such integration would enable researchers to upload a proteome of interest, generate the corresponding database online, and subsequently download it for local execution of Phylomizer, facilitating the reconstruction of species-specific phylomes in a local environment.



Fast Species Tree: Fast and Scalable Species tree Inference

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POSTER SESSION 3 - **P03.174**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The generation of species trees from genomic data is an important first step in understanding evolutionary relationships across the tree of life. With the ever increasing availability of genomic data, tree inference methods that can easily and rapidly produce accurate species trees are required to enable downstream analyses of these resources. Common approaches to species tree generation, including concatenated alignments or to combine individual gene family phylogenies, can require significant computational costs pre-processing the genomic data, inferring orthogroups, gene trees and performing phylogenetic inference. Here we present Fast Species Tree, a tool which aims to provide researchers with a rapid, scalable method of generating accurate species trees with limited prior requirements. Fast Species Tree leverages single-copy orthologs to build a concatenated sequence profiles for a set of input organisms, through the use of universal single-copy orthologs from BUSCO and OrthoDB. Rapid pseudo-multi-sequence alignments are constructed using an indexed-based concatenation of DIAMOND

sequence similarity search results. These concatenated pseudo-multiple-sequence alignments are then passed to a tree building software to infer the species phylogeny. Benchmarking Fast Species Tree on a range species datasets spanning the tree of life shows that Fast Species Tree is able to infer highly accurate species trees comparable to the best available methods. However, Fast Species Tree is also able to infer these phylogenies in substantially less time than comparative approaches. For example, Fast Species Tree can generating a phylogeny comprising more than 2000 bacterial species in less than 30 minutes on a standard desktop computer. While Fast Species Tree is suitable for species tree inference at all scales, it is designed to enable large scale sequencing projects improving the accessibility of large scale analyses for researchers without access to large scale compute resources. Fast species tree is written in python, executed as a single command and provides detailed output files to facilitate reproducibility.



From Sequence to Structure: Inferring the Origin and Evolution of Bacterial Motility

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POSTER SESSION 3 - P03.173, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Bacterial flagella represent one of the earliest forms of motility, enabling bacteria to propel themselves in a directed fashion. Flagella are powered by a multi-protein nanomachine that assembles into a membrane-spanning complex. Despite its crucial role in bacterial pathogenicity and biofilm formation, the early evolution of the flagellar proteins remains poorly understood, as traditional sequence-based methods often fail to resolve these ancient relationships.

In this study, we employ structure-informed, model-based phylogenetic analysis to explore the structural and functional diversification of flagellar proteins and trace the ancient evolutionary processes that gave

rise to bacterial motility. To overcome the limitations of sequence-based methods, particularly in the "twilight zone" where sequence similarity is highly decayed, we integrate 3Di structural characters and statistical models that account for sequence saturation.

Our findings reveal the remarkable diversity of flagellar proteins and their relatives and demonstrate how structure-informed phylogenetics can uncover evolutionary histories spanning billions of years. This approach offers new insights into the ancient molecular innovations that have shaped bacterial motility and its critical role in microbial evolution.



Inferring the deep past of Asgard archaea

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POSTER SESSION 3 - P03.176, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The phylum Asgardarchaeota is an archaeal group comprising a dozen classes of which only one includes cultured representatives. It is of specific evolutionary interest because eukaryotes likely sprouted from within this phylum. Previous studies have shown a wide diversity of (inferred) lifestyles, cell sizes and genome sizes of Asgard archaea. Recurrent adaptations to similar environments, such as high temperatures, may underlie the convergent evolution of sites towards certain amino acids. The artefactual grouping of taxa with a similar compositional bias has been shown to hinder a reliable inference of the Asgard archaeal phylogeny. In this study, we

use the expanding repertoire of Asgard archaeal genomes and apply recently developed models and a novel tool to mitigate compositional bias in order to obtain a robust species tree. In addition, we perform genome-wide phylogenetic analyses and reconcile the resulting gene trees with potential species trees to test the reconciliation likelihoods of different species tree hypotheses. These analyses provide an evolutionary framework for reconstructing ancestral genome contents and the evolutionary genome dynamics of Asgard archaea.



Machine learning phylogenetics

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POSTER SESSION 3 - **P03.171**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Understanding the relatedness of organisms is a fundamental question in evolutionary biology, often addressed through the construction of phylogenetic trees. Currently used methods for phylogenetic tree reconstruction face challenges with computational inefficiency due to ever-increasing genomic datasets and complex evolutionary models to analyse the data. Machine learning approaches can tackle these limitations. In prior work, we demonstrated that neural networks trained on site pattern frequency distributions can predict quartet topologies with an accuracy comparable to the maximum likelihood method while being significantly faster [1]. Here, we provide new evidence supporting the application of machine learning in phylogenetic tree reconstruction by

comparing alternative machine learning methods such as gradient boosting, length-informed networks as well as faster and more accurate inferences through the use of dimensionality reduction techniques.

References:

[1] Kulikov N., Derakhshandeh F., Mayer C., 2024. Machine learning can be as good as maximum likelihood when reconstructing phylogenetic trees and determining the best evolutionary model on four taxon alignments. *Molecular Phylogenetics and Evolution*.

<https://doi.org/10.1016/j.ympev.2024.108181>



New Deep-Branching Environmental Plastid Genomes and Their Implications for Plastid Evolution

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POSTER SESSION 3 - P03.175, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Eukaryotic algae are essential to the success of life on earth, forming the basis of many food webs. Yet, we still have a limited understanding of their convoluted evolution by endosymbiosis. Multiple hypotheses have been proposed, many involving successive endosymbioses between eukaryotes over vast evolutionary distances, but a clear consensus is lacking due to limited empirical evidence and sparse genomic data across the algal tree. To address this gap, we recently performed a phylogeny-guided, plastid genome-resolved metagenomic survey of Tara Oceans expeditions, recovering nearly 700 new non-redundant plastid genomes of environmental pelagic algae. Among these were four genomes, including one near complete, forming a deep-branching plastid lineage of globally distributed, nano-sized algae that we

informally named leptophytes. This lineage includes the previously uncharacterized marine plastid group DPL2, for which only 16S amplicon data had been available. Using 93 plastid genes and mixture models, including site-heterogeneous and site-and-branch heterogeneous models, we robustly place leptophytes alongside haptophytes and cryptophytes, though the precise relationships among them remain unresolved. Furthermore, we discuss the implications of our findings for the monophyly vs polyphyly of complex red plastids, a subject of renewed debate. Overall, our study demonstrates that metagenomics can reveal currently hidden diversity of organellar genomes, enabling complex phylogenomic analyses, and shows the importance of including this diversity to improve models for plastid evolution.



Testing the adequacy of amino acid substitution models at different time scales

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POSTER SESSION 3 - **P03.177**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

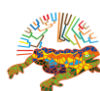
Molecular phylogeneticists propose hypotheses about the evolutionary relationships among lineages in the form of a phylogenetic tree, applying substitution models to the data at hand. The better a substitution model matches the real substitution patterns, the more accurate is the phylogenetic inference.

While relative model selection tests – comparison of the fit between available models– have become a standard step in a phylogenomic study, tests of absolute model fit remain relatively unexplored. Here we employ simulations to test model adequacy of different substitution models belonging to different amino acid substitution model families: site-homogeneous LG+G, finite mixtures LG+C60+G, Poisson+C60+G and EDM64+G, posterior-mean site frequencies PMSF-CAT+Poisson and infinite mixture Poisson+CAT.

We tested the performances of such models in describing features of the real datasets at

different time scales, as well as tested whether there is a correlation between model performances and divergence times or not. To do so, we analysed eight dataset of near-universally single copy orthologues (BUSCO) representing groups of taxa whose crown group diverged at different times (Mammalia, Vertebrata, Stramenopiles, Arthropoda, Metazoa, Viridiplantae, Eukaryota and Archaea).

Overall, our results found that site-heterogeneous mixture models better describe real datasets, largely agreeing with recent studies that found similar results. Within mixtures, we did not find a clear pattern, with models behave differently based on the dataset analysed. Finally, we advocate for the inclusion of model adequacy test (absolute fit) when preparing the conceptual design for a phylogenomic study.



The Gordian Knot of Interphylum Spiralian Relationships

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POSTER SESSION 3 - P03.170, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Among the many open questions in animal phylogenetics are the interphylum relationships of spiralian. While the membership, if not necessarily the clade name, of Spiralia has been relatively stable ever since the lophophorates were shown to not be Deuterostomes, the relationships between the constituent phyla have not. We have focussed our efforts on untangling the relationships between the five largest phyla (Annelida, Brachiopoda, Mollusca, Nemertea and Platyhelminthes). We find support in the literature for all 15 possible unrooted trees that can relate these five phyla. This lack of clarity has important implications for our understanding of the clade's history and for the evolution of traits as biomineralization, segmentation, larvae, etc. We have used two recent phylogenomic datasets to explore possible causes of this remarkable example of taxonomic instability. Using a combination of taxon-

jackknifing and bootstrapping on empirical and simulated data, we explored the support for all (105) rooted and (15) unrooted 5-phylum spiralian topologies under site-homogeneous and site-heterogeneous substitution models. We found marginal preference for the same 5-phylum topology in the vast majority of our unrooted analyses. Our rooted analyses, however, suggested that preference for rooting Spiralia on Platyhelminthes is due to a long-branch artefact between this clade and Gnathifera. Our results also showed that the interphylum spiralian branches were even shorter, on average, than the notoriously short Deuterostome branch. Combined, these two lines of evidence suggest that efforts to resolve spiralian relationships convincingly will have to address systematic errors arising from branch-length and site-compositional heterogeneity, as well as errors linked to outgroup rooting.



An independent phylogenomic dataset sheds new light on the eukaryotic Tree of Life

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MEETING ROOM 131, August 22, 2025, 10:30 AM - 12:30 PM

Understanding the phylogenetic relationships between all known forms of life remains a major challenge. Molecular phylogenetic analyses using large sets of highly conserved protein-coding genes for the reconstruction of the Tree of Life have become the norm. In the case of eukaryotes, this increasing use of phylogenomics (multi-gene phylogenetic analyses) has grouped most of the eukaryotic diversity into a small number of monophyletic so-called supergroups (e.g., Amorphea, Archaeplastida). Nevertheless, the deepest relationships among these supergroups remain uncertain and, furthermore, some new phylum-level 'orphan' eukaryotic lineages continue to be discovered, such as Hemimastigophora or Provora. Most phylogenomic studies relied on very similar datasets of core marker genes to infer the eukaryotic Tree of Life. In this work, we aim at testing an independent marker gene

collection to infer such deep relationships among major eukaryotic lineages using both Maximum Likelihood and Bayesian Inference with an improved and balanced taxon sampling. We gathered a collection of 264 proteomes spanning the entire characterized eukaryotic diversity and reconstructed their phylogeny using 354 proteins derived from the BUSCO dataset, which are well conserved and supposed to have been vertically inherited. We applied several strategies to alleviate potential artefacts due to long branch attraction and/or compositionally biased taxa and assessed the robustness of inter-lineage relationships using state-of-the-art phylogenetic analyses and tests. I will present how our analyses contrast with previous ones, in particular regarding deep relationships not previously observed and how they impact our understanding of the early diversification of eukaryotes.



Deciphering the origin and timing of major waves of gene acquisition unravel complex interactions underlying the origin of eukaryotes

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MEETING ROOM 131, August 22, 2025, 10:30 AM - 12:30 PM

The origin of eukaryotes remains a central enigma in biology. Eukaryotes are fundamentally different in their cell organization and structural complexity from their prokaryotic counterparts, a leap in complexity unparalleled by any other transition of a similar age. Ongoing debates agree on the pivotal role of a symbiosis between an alpha-proteobacterium and an Asgard archaeon—yet the nature, timing and contributions of other potential bacterial partners, or the role of interactions with viruses, remain contentious. The unveiling of the timing of events in eukaryogenesis has historically been hindered by the deep evolutionary time in which this transition occurred, the long branch between the Last Eukaryotic Common Ancestor and prokaryotes, and the lack of any other transition of similar magnitude having ever occurred.

The breadth of new genomic knowledge of diverse, sometimes early-branching eukaryotic lineages, coupled with a

refinement of phylogenetic inference methods, has created an unprecedented momentum to tackle this fundamental question in the origin of one of the major divisions of cellular life. We employed state-of-the-art phylogenomic approaches to reconstruct gene trees across comprehensive datasets covering the known diversity of cellular life and viruses, allowing us to provide an updated reconstruction of the last eukaryotic common ancestor (LECA) proteome, and the phylogenetic origin of each of its constituent protein families, highlighting major contributors beyond host and endosymbiont to give rise to eukaryotes. Additionally, we employed robust methods based on these gene phylogenies to trace the relative timing of these key players to the LECA protein repertoire. Altogether, our results suggest ancient eukaryotes originated within complex microbial ecosystems through a succession of symbiotic interactions that left a footprint of horizontally transferred genes.



Large-scale phylogenomics nests ten additional phyla-rank lineages in the PVC superphylum

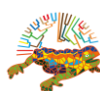
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MEETING ROOM 131, August 22, 2025, 10:30 AM - 12:30 PM

The Planctomycetota-Verrucomicrobiota-Chlamydiota (PVC) superphylum is a well-supported bacterial clade encompassing several phyla markedly diverse with respect to morphology, metabolism and lifestyle. Members of these phyla have attracted significant research interest due to distinctive features such as endosymbiosis, diverse cell-division modes, and atypical bacterial traits like internal compartmentalization, sterol biosynthesis, and phagocytosis-like prey engulfment. The aggregation of these traits within a single monophyletic clade makes the PVC superphylum a unique framework for investigating their evolutionary origins. This effort necessitates the robust reconstruction of phylogenetic relationships within the superphylum. Advances in next-generation sequencing and metagenomics have greatly expanded our understanding of the Tree of Life, leading to significant shifts in the PVC topology, including the emergence of novel candidate lineages. However, studies using different phylogenomic datasets and methods often yield conflicting topologies, highlighting the need for a more comprehensive and rigorous phylogenomic analysis. To address this, we screened the global bacterial diversity archived in NCBI,

aiming to identify lineages phylogenetically related to the PVC superphylum. Utilizing multiple sets of phylogenetic markers in an expanded taxon sampling of PVC and related bacteria, we applied elaborate phylogenomic approaches to reconstruct the evolutionary relationships within this group. Our analyses reveal ten additional phylum-level lineages, deeply branching within the PVC superphylum and tentatively quadrupling the number of recognized PVC phyla. Among these findings, we report a previously unrecognised sister lineage to Chlamydiota and reveal new branching patterns within the superphylum. Members of these ten additional PVC lineages have been recovered from diverse environments such as deep-sea hydrothermal vents, anaerobic digesters, wastewater systems or host-associated environments. Metabolic reconstruction suggests that most representatives are anaerobic, motile and have a heterotrophic lifestyle. Ongoing analyses aim to further characterize their environmental distribution and abundance, as well as to explore their genomic potential. This work will provide deeper insights into their metabolic capabilities, mechanisms of cell division, and possible adaptation to host-associated lifestyles.



Solarion arienae is rare microbial relict from newly described eukaryotic supergroup

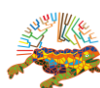
Valt M¹, Pánek T¹, Mirzoyan S¹, Tice A^{2,3}, Jones R², Dohnálek V⁴, Doležal P⁴, Mikšátko J⁵, Rotterová J^{1,6}, Hrubá P¹, Brown M^{2,7}, Čepička I¹

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MEETING ROOM 131, August 22, 2025, 10:30 AM - 12:30 PM

During the last decade, our understanding of eukaryotic evolution has increased immensely. The eukaryotic tree of life has been reworked multiple times since the advent of molecular phylogenetic era, often by discovery of novel deep branching protist lineages. A novel protist, *Solarion arienae* presented here, is such a novel deep lineage. *Solarion* is a morphologically inconspicuous protist that is rare in environmental libraries. We assign it to the new phylum, where together with *Provora*, *hemimastigophoreans*, and *Meteora*, they form a new eukaryotic supergroup. The organism has two cell types, a flagellated and a “sun-like” form. The latter displays a novel type of extrusomes we have examined

by electron tomography. Deep-branching protist lineages are thought to be indispensable for unraveling the early evolution of mitochondria, because they frequently retain metabolic pathways that have been lost in model organisms. *Solarion* is another such as it possesses mitochondrially-encoded *SecA* gene, a remnant of an ancestral alphaproteobacterial protein secretion pathway, that has been almost entirely lost in extant mitochondria. The discovery of *S. arienae* broadens our understanding of early eukaryotic evolution and facilitates the study of proto-mitochondrial metabolic remnants, shedding light on the complexity of ancestral eukaryotic life.





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**S41 - Post-phylogenomics: new and
evolving molecular methods to address
challenging phylogenies**



A timetree of Fungi dated with fossils and horizontal gene transfers

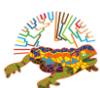
Szánthó L^{1,2,9}, Merényi Z³, Donoghue P⁴, Gabaldón T^{5,6,7,8}, Nagy L³, Szöllősi G^{1,2,9}, **Ocana-pallares E**^{5,6,9,10}

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POSTER SESSION 1 - P01.326, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Dating the tree of Fungi (ToF) has been challenging due to a paucity of fossil calibrations and its broad taxonomic diversity. We reconstructed and dated a comprehensive ToF comprising 110 fungal species, utilizing 225 phylogenetic markers and accounting for site-specific amino acid compositional heterogeneity. To address uncertainties in fungal dating, we sampled chronograms from four relaxed molecular clock analyses, each integrating distinct sets of calibrations and relative time-order constraints. The first analysis employed a core set of 27 calibrations alongside 17 relative constraints derived from fungi-to-

fungi horizontal gene transfer events. The other three analyses extended this core set with additional timing information identified in our re-evaluation of pectin-specific enzymes' evolution in Fungi. Our summary timescale, integrating analytic uncertainties, suggests older ages for crown-Fungi (1401–896 Ma) than recently reported, aligning with recent discoveries of ancient fungal-like fossils. Our study offers a refined timescale of the diversification process of Fungi, providing also a minimum age for ancient interactions involving fungi and pre-embryophyta algae in terrestrial ecosystems (1253–797 Ma).



Amino Acid Exchangeabilities Differ More Between Surface and Buried Sites than Among Species

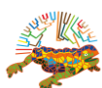
Goodman P¹

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POSTER SESSION 1 - **P01.329**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Evolutionary Biologists capture the process of molecular evolution using substitution models, which represent the likelihood or rate of change at specific sites in the DNA sequence. These models are essential components of maximum likelihood and Bayesian methods for phylogenetic inference, as improved substitution models lead to more accurate phylogenies and branch length estimates. The rate matrix, Q , comprises exchangeability values, an abstract parameter representing the relative substitution rate between amino acids, and the amino acid frequencies. Previous work has shown improvements in substitution models that account for protein structural features, but whether this improvement is driven by unique structural exchangeability values or different amino acid frequencies across structural regions is unclear. We used

recent developments in computational tools for substitution model inference and data availability for protein structure to investigate the impact of protein structure on amino acid exchangeability values. Using predicted protein structures from the AlphaFold database, we partitioned orthologous genes from four taxa into exposed and buried residue sections based on their solvent accessibility, and trained Q matrices on each taxon-structure combination. The exchangeability values derived from these partitioned Q matrices differed more between accessible surface area than between taxon, highlighting the greater impact of protein structure on substitution patterns. These results underscore the potential for structure-aware substitution models to enhance phylogenetic inference.



Diversification of allotetraploid species with identical genomic composition

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POSTER SESSION 1 - **P01.332**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Polyploidy, the condition of possessing more than two complete sets of chromosomes, plays a crucial role in plant evolution. Among its various forms, allopolyploidy, resulting from hybridisation between different species, has proven to be a particularly influential evolutionary mechanism. Allopolyploids may have inherited variability from their ancestors through multiple independent origins; in such cases, their genetic diversity reflects that of the parental lineages involved in separate hybridisation events. Alternatively, some polyploid organisms arise from a single hybridisation event and subsequently diversify into multiple polyploid taxa. Distinguishing between these scenarios is essential for understanding the evolutionary dynamics of polyploid species. However, it is often challenging due to complex patterns of post-polyploidisation genome evolution, such as subgenome homogenisation and gene loss.

The genus *Chenopodium* L. sensu stricto comprises ~150 species distributed worldwide. It consists of eight basic diploid lineages (A-H), which have often combined through hybridisation to form allopolyploid species. One allopolyploid combination (CCDD) stands out for its abundance and diversity. Recognised as several distinct species based on their morphology, ecological specialisation and geographic distribution, CCDD tetraploids are a valuable model for studying the genomic and evolutionary processes driving allopolyploid diversification. We investigated their origin by testing the single versus multiple origin hypotheses to provide a broader framework for studying allopolyploid evolution across taxa.

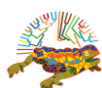
Materials and methods: To reconstruct the evolutionary history of CCDD tetraploids, we

performed whole-genome re-sequencing on 140 individuals sampled across the geographic and taxonomic range of the group.

Previous studies have shown that the distinct genomic contributions of ancestral lineages are largely conserved in these allopolyploids. This allows straightforward phasing of subgenomes. As a result, tools originally developed for diploids, such as sNMF for population structure analysis, IQ-TREE for phylogenetic inference and SMC++ for demographic modelling, were effectively applied to each subgenome separately, allowing comparative analyses.

Results: The integration of high-resolution data allowed a more precise species delimitation, revealing a novel species that had not been distinguished in previous classifications. Phylogenomic analyses revealed that the C and D subgenomes form highly consistent groups, suggesting a common evolutionary history. The demographic histories of both subgenomes were also harmonised, with comparable patterns of population sizes. The congruence of phylogenomic and demographic signals between the subgenomes suggests a single origin of the CCDD tetraploids.

Conclusion: Through the integration of phylogenomic and demographic analyses, our results demonstrate that the combination of complementary analytical approaches can effectively dissect the complex origins of allopolyploid species. Here, the consistent patterns support the single origin hypothesis for the CCDD tetraploids. Our study not only contributes to a more nuanced understanding of polyploid evolution in *Chenopodium* but also provides a scalable framework for dissecting polyploid ancestry in other taxa.



Inferring gene flow from phylogenies with too many genomes

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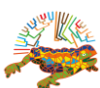
POSTER SESSION 1 - P01.334, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Gene flow is now widely recognized as a central force in shaping species evolution, but despite its importance, it remains challenging to quantify across lineages. This task becomes even more complicated with confounding processes, such as ancient gene flow and incomplete lineage sorting, making it harder to reconstruct species history. Nonetheless, recent advances in sequencing technologies provide extensive genomic data needed to revisit these questions at scale. By integrating both within-species and between-species sequence variation, we aim to disentangle the contribution of gene flow and other short and long-scale evolutionary processes.

We introduce a new model of species evolution with gene flow (FlowT) that incorporates multiple interacting evolutionary forces within a phylogenetic

framework, such as mutation bias and genetic drift, facilitating analysis at evolutionary timescales where gene flow is understudied. FlowT leverages the observed allele frequencies, making it well-suited for large-scale genomic datasets. Additionally, because it automatically integrates all the possible genealogies to estimate the species tree, it avoids the computational burden of traversing the whole genealogical space.

To assess the performance of our model, we simulated multiple sequence alignments across a range of evolutionary scenarios. Using Bayesian inference, we accurately recover the mutation, gene flow rates, and divergence times. As such, these results demonstrate the ability of our model to infer gene flow across phylogenetic timescales from large-scale genomic data.



PhyloFusion - Fast and easy fusion of rooted phylogenetic trees into rooted phylogenetic networks

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POSTER SESSION 1 - P01.330, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

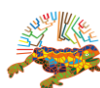
Phylogenetic trees typically represent the evolutionary relationships between a set of organisms. However, in evolution, reticulate events such as horizontal gene transfer, speciation by hybridization, and incomplete lineage sorting play a significant role. Rooted phylogenetic networks are more effective at accurately illustrating evolutionary histories in such cases. Although unrooted networks are well studied, recent methods for the fast and easy calculation of rooted networks remain scarce, despite their importance. Recent developments have aimed to address this gap. Recently, Zhang et al. (2023) introduced a new algorithm, ALTS, for computing a rooted phylogenetic network from a set of rooted phylogenetic trees. Here, we present PhyloFusion, an algorithm that extends ALTS by supporting trees with unresolved nodes and missing taxa. PhyloFusion is a fast and user-friendly method for constructing rooted networks that preserve all trees in the input set.

The algorithm constructs a tree-child network by first selecting a fixed total ordering of the full taxon set. Based on this ordering, internal nodes in each input tree are labeled, resulting in lineage taxon string (LTS) encodings for each taxon. For each taxon, the shortest common supersequence (SCS) of its LTSs across all input trees is determined using a profile alignment heuristic. To address the algorithm's preference for hard display over soft display, two preprocessing heuristics are applied at the beginning of each recursive call to minimize hybridization events. These shortest common supersequences are then decoded to build the

network structure while aiming to further limit the number of reticulate events. In the resulting tree-child network, every internal node has at least one child that is a tree node, ensuring full compatibility with the input trees. To explore the space of possible networks, the algorithm applies a greedy heuristic that considers a subset of taxon orderings and computes shortest common supersequences, potentially yielding multiple networks with identical hybridization scores.

We implemented and evaluated the PhyloFusion algorithm within the SplitsTree app, making it suitable for the interactive exploration of input tree sets. Potential applications include sets of functionally related gene trees and segmented RNA viruses, where segmentation results in topological differences among trees. Although the algorithm operates efficiently on tens of trees and hundreds of taxa, it is most effective when the number of reticulate events remains limited to maintain interpretability. In a comparative study against two alternative heuristics, PhyloFusion demonstrated competitive performance in terms of both runtime and the number of hybridization events.

In conclusion, we present PhyloFusion and demonstrate its use in exploring the application of rooted phylogenetic networks for merging collections of rooted phylogenetic trees into a comprehensive network. Its integration into SplitsTree makes it a practical tool for biologists to explore evolutionary scenarios involving reticulate events.



Population genomic divergence reveals uncertainties in species identification of cave-dwelling groundwater isopods

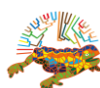
Lapadula S¹, Manenti R¹, Barzaghi B¹, Ficetola G^{1,2}, Sherpa S¹

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POSTER SESSION 1 - P01.328, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Biodiversity conservation is advancing, yet much of nature remains unexplored. Still off the map, subterranean environments hold a significant portion of the world's biodiversity. Most of our knowledge about cave biodiversity remains limited to faunistic checklists, for which the delimitation of species boundaries still relies on morphological characters. However, cave biology still lacks genomics research to improve taxonomic identification, clarify species evolutionary history, and support conservation efforts. Here, we examined genome-wide diversity of seven cave-dwelling *Monolistra* isopod species throughout the pre-alpine arch in Italy and Switzerland. We used genome-wide single nucleotide polymorphisms obtained through double digest restriction-site associated DNA sequencing to reconstruct phylogenetic relationships and infer population structure. Phylogenetic tree reconstruction revealed

strong divergence among the 21 cave populations studied, splitting into seven phylogenetic clades. Both species and gene trees inferred with a multispecies coalescent model confirmed an east-west clade divergence, yet highlighted major conflicts between morphology-based taxonomy and genomic data. Population structure analyses further identified 11 genetic groups and genetic isolation between each cave system, with varying level of within-clade diversity. Our findings have crucial taxonomic implications, and accurate species delimitation will require further comprehensive analyses. Our study implies that the true level of diversity within the genus *Monolistra* could be much greater than currently understood, and therefore stresses the importance of genomics research to improve conservation strategies for subterranean biodiversity.



Resolving taxonomic uncertainties in genus of the edible chayote (*Sechium* P. Browne (Cucurbitaceae)) and exploring potential center of domestication of chayote through genomics analysis

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POSTER SESSION 1 - **P01.333**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

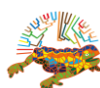
Background: The genus *Sechium* (Cucurbitaceae), was historically considered monospecific, including only *S. edule* ssp. *edule* (the edible chayote), but has recently undergone significant taxonomic revisions. Numerous Mesoamerican species were initially placed in different genera, but have been recently reassigned to *Sechium*. The recognized number of species in the genus has fluctuated. For instance, recent studies suggest that both *S. chinantlense* and *S. compositum*, once considered separate species, may instead be subspecies of *S. edule*. This challenges conventional classifications and remains debated. Additionally, the domestication history of *S. edule* remains unclear due to the perishable nature of its seeds and the lack of direct archaeological evidence.

Materials and Methods: To address taxonomic uncertainties, we used SNP (Single Nucleotide Polymorphism) analysis, which is effective for understanding adaptive processes and resolving recent plant radiations, due to their rapid evolutionary rate, and for the evaluation of genomic diversity. We applied multispecies coalescent (MSC) methods for species delimitation. Our study included 79 individuals from species across their geographic range in Mexico: four wild taxa (*S. chinantlense*, *S. compositum*, *S. hintonii*, *S. edule* ssp. *sylvestre*) and one cultivated subspecies (*S. edule* ssp. *edule*). The selection of samples focused on three objectives: inferring their phylogenetic relationships, admixture assessment, and population genomic analysis. Due to computational constraints, we reduced the dataset to 40 individuals for phylogenetic analysis. For structure analysis, 76 samples were used, and 69 samples for population genetics. To refine divergence time estimates, the analysis of the *Sicyos* group-- including *Sechium*--, was

updated with mitochondrial and ribosomal DNA sequences from multiple studies, covering a total of 61 species.

Results: Our results supported that *S. edule* ssp. *edule*, *S. chinantlense*, *S. compositum*, and *S. hintonii* are distinct species, contrary to prior studies suggesting *S. chinantlense* and *S. compositum* were subspecies of *S. edule*. Genetic differentiation analyzed using SNP data and *F_{ST}* values, reinforced these species distinctions. Low levels of hybridization were detected in some individuals, but these were minimal. The earliest diverging species in the *Sechium* group is *S. hintonii*. Divergence times inferred from *rcbL*, ITS sequences, and SNPs indicate a more recent separation between the taxa than previously estimated, and in particular *S. edule* ssp. *edule*, *S. chinantlense*, and *S. compositum* are closely related species that diverged recently. *Sechium edule* ssp. *sylvestre* was genetically distinct from cultivated varieties, supporting its classification as a separate entity.

Conclusion: This study advances in the understanding of the evolutionary relationships within *Sechium*, highlighting the distinctiveness of species previously considered subspecies. SNP analysis and MSC methods provided robust insights in the phylogeny and divergence times, suggesting a recent split between *S. edule* ssp. *edule* and its relatives. The observed genetic structuring and range reductions, coupled with the potential threat of climate change, underscore the include need to include the species, varieties and cultivars of the genus in conservation programs, like the IUCN Red List. Given ongoing habitat loss and limited distributions, proactive strategies will be essential to protect the genetic diversity of this important genus.



SARS-CoV-2 Zoonotic and Reverse-Zoonotic Transmissions Between Humans and Mink

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POSTER SESSION 1 - **P01.327**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The virus Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a zoonotic pathogen, able to be transmitted back and forth between humans and mink. This cross-species transmission has been, and still is today, cause for concern, as it potentially enables the virus to escape antibody neutralization or vaccine protection.

Variants of SARS-CoV-2 that were initially found on Danish mink farms, were later found among the human population of Denmark. These variants were claimed to have lower susceptibility to antibody neutralization, ultimately resulting in the culling of 17 million mink in Denmark.

This work investigates what underlies this zoonotic and reverse-zoonotic ability. This is done by (I) analysing genomic SARS-CoV-2 sequences, obtained from both humans and mink in Denmark, (II) performing a phylogenetic and transmission analysis to identify changes over time in the DNA sequences and the corresponding amino acid sequences of the SARS-CoV-2 spike protein, (III) comparing the structure and sequence human and mink Angiotensin Converting Enzyme 2 (ACE2) receptors, (IV) modelling amino acid changes into the three-dimensional structure of the SARS-CoV-2 spike protein, followed by (V) simulations of the interaction between the spike protein and the ACE2 receptor of both hosts, and finally (VI) analysing the impact of these mutations by measuring, among others, the protein interaction and stability.

Some of this work is done in overlap with a paper on the emergence and spread of SARS-CoV-2 variants between humans and farmed mink in Denmark, as published in PLOS

Pathogens

doi:

10.1371/journal.ppat.1012039.

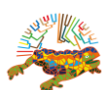
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<https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1012039>)

This paper investigates the occurrence of zoonotic and reverse zoonotic transmission events between humans and mink in Denmark. These calculations were part of this work as well. Additionally, analyses of this work were used as a starting point for analyses in the paper.

Several variants of SARS-CoV-2 were identified in this work as being able to transmit back and forth between humans and mink. For each of these variants, an in-depth analysis of the protein properties was performed in efforts to explain the occurrence of certain amino acid changes and distinguish a pattern or commonality. One of the identified variants is known as the Cluster 5 variant, carrying, among others, the D614G and Y453F mutations. This variant is known to undergo zoonotic and reverse-zoonotic transmission. This work identified three additional variants, able to do the same with a higher degree of efficiency, which are not reported in literature as of yet.

This work also draws attention to the origin of SARS-CoV-2, by running simulations of the circulating variants in interaction with different host ACE2 receptors. From these simulations it becomes clear that the occurring mutations are detrimental for the interaction with the mink ACE2 receptor, but beneficial for the interaction with the human ACE2 receptor. Furthermore, these mutations appear to be neutral in their interaction with the ACE2 receptor of a range of hosts, such as bats and pangolin.



Transcriptomic insights into the enigmatic origins of monotypic genera *Mankyua* and *Helminthostachys* in Ophioglossaceae

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POSTER SESSION 1 - **P01.331**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The Ophioglossaceae, one of the earliest-diverged lineages of monophytes, is characterized by large genome sizes, high chromosome numbers, and numerous polyploid species. The phylogenetic relationships among the four genera (*Botrychium*, *Ophioglossum*, *Mankyua*, and *Helminthostachys*) that constitute the fern family have not yet been elucidated, especially the evolutionary origins of the two monotypic genera, *Mankyua* and *Helminthostachys*, remain unresolved. The endangered Jeju *Mankyua chejuense* is an endemic species to Gotjawal, a unique type of forest found on Jeju Island, South Korea, developed over uneven, porous lava flows which create a rugged landscape with limited soil depth. In contrast, *Helminthostachys zeylanica* is widely distributed across South Asia and Australia. Previous studies utilizing chloroplast markers have yielded conflicting topologies depending on the dataset used, particularly with regard to the phylogenetic placement of *Mankyua* and *Helminthostachys*. This suggests that Ophioglossaceae has a complex evolutionary history that includes the possibility of hybridization, and highlights the need for nuclear genome-scale data to elucidate the causes of these discrepancies.

We conducted a phylogenomic analysis based on low-copy nuclear genes derived from transcriptome assemblies, focusing on *Mankyua* and *Helminthostachys* to elucidate their evolutionary relationships and assess evidence of reticulate evolution. Our sampling included 14 individuals: two representatives each from six ingroup species (*Botrychium japonicum*, *B. lunaria*, *Mankyua chejuense*, *Helminthostachys zeylanica*, *Ophioglossum vulgatum*, and *O. pendulum*), and two outgroup samples from *Psilotum*. We performed de novo assemblies for six individuals and incorporated eight

transcriptomes from NCBI. A total of 384 low-copy orthologs, identified using OrthoFinder, were codon-aligned for downstream phylogenomic analyses. Phylogenies were reconstructed using concatenation (IQ-TREE) and coalescent (ASTRAL) methods; reticulate networks were inferred with PhyloNet, and hybridization was tested using HyDe.

In IQ-TREE analyses, although bootstrap values were consistently high (>95%), gene concordance factors at the nodes uniting *Mankyua* and *Helminthostachys* were notably low (39.8% and 20.3%), indicating substantial conflict among gene trees. ASTRAL species trees similarly revealed topological instability involving these taxa, with low quartet support at key nodes. PhyloNet analyses demonstrated substantial improvements in network log-likelihoods when incorporating reticulation events, confirming the presence of reticulation. HyDe analyses detected significant hybridization signals involving *Mankyua* and *Helminthostachys*, with contributions from both *Botrychium* and *Ophioglossum*. These results suggest that strictly bifurcating tree models may inadequately represent the evolutionary history of these taxa, and instead support a scenario of reticulate processes.

This study represents one of the first empirical efforts to resolve longstanding phylogenetic discordance within Ophioglossaceae using transcriptome-based nuclear markers. Our analyses indicate that the evolutionary relationships among the four genera, including *Mankyua* and *Helminthostachys*, have likely been shaped by complex reticulate processes. By applying low-copy nuclear orthologs, we propose a practical phylogenomic strategy for elucidating reticulate evolutionary patterns in early-diverging plant lineages, which have often been challenging to resolve using traditional bifurcating models.



CASTER: Direct species tree inference from whole-genome alignments

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MEETING ROOM 131, August 18, 2025, 10:30 AM - 12:30 PM

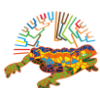
Introduction: With the rapid availability of new genomes, phylogenetics has gained an opportunity to infer not only more accurate species-level relationships (species trees) but also the heterogeneity of evolution across the genome (gene trees). However, available methods are lacking. The dominant approach, concatenating all genomic loci, fails to account for discordance across the genome, leading to statistical bias. An alternative widely-used approach infers gene trees from individual loci and then combines them. However, this two-step approach struggles with defining loci that are both short enough to avoid recombination and long enough to prevent high error rates per tree. Bayesian methods that co-infer gene trees and species trees exist but are not scalable. Another promising approach has been inferring the tree from all sites while accounting for discordance, but these site-based methods have shown limited scalability and accuracy. As a result of these limitations, genome-wide analyses are forced to sample loci instead of using the entire genome as input.

Rationale: We developed a site-based method called CASTER to enable truly genome-wide phylogenomic analyses. CASTER is based on the idea that each site in the alignment can "vote" for or against each tree topology for four species (a quartet), with the tree receiving the maximum total vote across sites and quartets winning. CASTER has four key features: 1) It provides theoretical statistical guarantees under the multi-species coalescent model of incomplete lineage sorting, coupled with several sequence evolution models, without requiring costly likelihood calculations. 2) It is extremely scalable (linear in the number of sites and roughly quadratic in the number of species), allowing for the analysis of entire mammalian genomes (1.8 billion sites) across 241 species in 30 hours using 512GB of memory. 3) It eliminates the need for arbitrary

locus delineation. 4) It produces a score for each genomic position that, when summarized across windows, reveals changes in evolutionary histories across the genome. CASTER can handle simpler nucleotide models using patterns of sites (CASTER-site) or more complex models using pairs of sites (CASTER-pair).

Results: Comparing CASTER to several alternative methods representing the current best approaches, we demonstrated through simulations that CASTER outperforms these methods in terms of scalability and is the most accurate under most conditions tested. Our simulations included a range of realistic biological processes, including recombination, which is often missing from previous studies, and incomplete lineage sorting. CASTER-site and CASTER-pair handled 2 and ½ billion sites, resp., given only 64 cores, 128GB of memory, and up to two days of running time. CASTER scores correlate well with the amount of gene tree discordance, and its estimates of statistical uncertainty are accurate. On mammalian and avian genome-wide datasets, we demonstrate that CASTER can analyze the entirety of the data, generate accurate trees, and produce scores that reveal heterogeneities across the genome. Deviations in CASTER scores from our expectations indicated errors in input alignments, instances of rapid radiation among species, and cases where evolution appears reticulate.

Conclusion: CASTER enables phylogenetic analyses that include all the sites in a genome, modeling the heterogeneity of rates and topologies across the genome. It requires no demarcation of loci and provides interpretable outputs that help biologists understand not only the species relationships but also the mosaic of evolutionary histories across the genome.



Paralogy, orthology inference biases, and extreme discordance in plant phylogenomic data sets driven by assembly artifacts and sequencing strategies

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MEETING ROOM 131, August 18, 2025, 10:30 AM - 12:30 PM

Target enrichment and genome skimming have become the most common sequencing approaches for phylogenomics studies in plant systematics. Furthermore, several pipelines have been developed exclusively to facilitate these data sets' assembly and locus extraction. This has largely facilitated analysis of plant data sets, leading to routine discovery of gene tree discordance that has mainly been attributed to incomplete lineage sorting (ILS), hybridization, and even gene tree estimation error. While ILS and especially hybridization are common in plants and can explain the discordance patterns, little attention has been given to the effects of suboptimal assembly that inflates paralogy, and this, in turn, leads to orthology inference biases, wrong detections of whole genome duplications, and eventually to wrong hypotheses of discordance and hybridization. Here, we explore an example of target enrichment in *Amaranthaceae* (amaranth and quinoa family). *Caroxyloneae* and *Salsoleae* constitute a species-rich lineage of *Amaranthaceae* containing around 40 genera and 300–400 species, including the largely polyphyletic *Salsola*. Members of this lineage are primarily distributed in the steppes and deserts of Eurasia and northern and southern Africa. It includes leaf- and stem-succulent halophytic, xerohalophytic, xerophytic, and ruderal plants, where C4 photosynthesis is prominent. Generic boundaries in *Caroxyloneae* and *Salsoleae* have been historically a matter of long-standing debates, with numerous transfers, reinstatements, or newly established genera in recent years. To date, phylogenetic analyses of the tribe have been based on a limited number of DNA regions, leaving most phylogenetic relationships unclear in an already highly taxonomically challenging group. To build a comprehensive backbone phylogeny of the

group to clarify generic boundaries and investigate patterns of C4 photosynthesis, we used 483 putative single-copy loci from a custom target enrichment bait set and sampled 175 spp. from 36 genera from *Caroxyloneae* and *Salsoleae*. We recovered many paralogs for all species, including many pseudo-gene copies. These paralogs were spread across all *Chenopods* II, with several species even having gene copies as far as nested within *Polycnemoideae* (one of the other subfamilies in *Amaranthaceae*). This extreme paralog pattern largely halted the ability to infer orthologs using tree-based methods because the many duplications found in the gene trees resulted in mainly small orthologs, which ultimately resulted in a phylogeny with massive discordance and low to no support for most nodes. Here, we explore the challenges and strategies for overcoming the issue of rampant paralogy to attempt to build a backbone of *Caroxyloneae* and *Salsoleae*. We found that low assembly coverage and/or exon similarity will produce artifact contigs that inflate the “paralog” recovery and largely interfere with orthology and tree inference. Furthermore, tree-based orthology was biased in allopolyploidy cases (or similar patterns), resulting in the loss of entire clades in the orthologs. To fix this, *Caroxyloneae* and *Salsoleae* required assemblies that considered sequencing coverage (by sample and gene), using only closely related references (by clade) and high exon similarity. Still, *Caroxyloneae* and *Salsoleae* seem to be a complex group with a history of ancient hybridization and recent allopolyploid species. The patterns found here should be a cautionary tale for all target enrichment and genome skimming projects to be mindful of the locus assembly process.



Phylotranscriptomics as the Most Appropriate Tool for Resolving Complex Phylogenies: Insights from the *Ophrys* genus

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MEETING ROOM 131, August 18, 2025, 10:30 AM - 12:30 PM

Background: The orchid genus *Ophrys* is an outstanding example of an explosive and recent plant adaptive radiation. The rapid diversification of this genus, combined with its notable capacity for hybridisation, poses a significant challenge in addressing the phylogenetic relationships among its lineages. Dealing with a substantial number of nuclear markers is so necessary to accurately grasp the evolutionary history of this genus. However, the large size of its genome (5 to 7 gigabase pairs (Gb)) makes it impossible to resequence entirely on a significant number of individuals. Most of the phylogenetic hypotheses that have been published to date are limited to few loci, and either poorly resolved or prone to display gene trees incongruences.

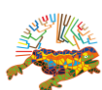
In this context, we decided to use phylotranscriptomics as a genome complexity reduction technique, to work at the genomic scale without sequencing the entire genome. This approach enabled us to obtain and compare thousands of gene trees to improve resolution and tease apart the relative contribution of introgression and Incomplete Lineage Sorting in *Ophrys* diversification.

Material and Methods: Flowers from representative species of eight of the ten *Ophrys* main lineages were sampled in the wild in southern France and sequenced with Illumina short read technology. Transcriptomes were then de novo assembled using a combination of several tools. We developed a whole pipeline to obtain an important set of about 8000 nuclear 1-1 orthologous genes, as well as chloroplastic genes from this transcriptome assembly. This orthologous genes were used to reconstruct a phylogeny of the *Ophrys* genus with an unprecedented resolution.

We implemented several introgression tests with different levels of complexity, using both gene trees topologies and branch lengths: from simple Patterson's D statistic to branch-length based approximate likelihood method and phylogenetic network inference by maximum pseudo-likelihood. Finally, in order to explore a putative adaptive role of introgression in *Ophrys* diversification, we explored the phylogeny of several key genes in *Ophrys* pollinator attraction and compared it with the one of the species tree.

Results: The phylogeny largely confirms the relationships between the main *Ophrys* lineages obtained by other recent methods but also provides new insights within each of these lineages and highlights strong discrepancies between gene trees for some part of the species trees. We show that this incongruence between gene trees is not only due to Incomplete Lineage Sorting (ILS). Although introgression has been hypothesised to play an important role in *Ophrys* evolution, we provide for the first time quantitative characterisation of this reticulate evolution at the genus scale. Indeed, our introgression tests were able to highlight several complex introgression events between *Ophrys* lineages as well as potential hybrid speciation.

Conclusion: Our results highlight the potential of phylotranscriptomics to address challenging phylogenies of groups such as the genus *Ophrys*, which presents a large genome with many discordances arising from both ILS and gene flow. They also show that the classical representation in a phylogenetic tree is not appropriate to represent the evolution of groups with highly reticulated evolution, demonstrating the importance of phylogenetic networks for these groups.



Unraveling evolutionary complexity in a young adaptive radiation of New Caledonian persimmons

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MEETING ROOM 131, August 18, 2025, 10:30 AM - 12:30 PM

Adaptive radiations are dynamic interplays between speciation, expansion and extinction, often starting from long distance migrations. It remains unclear whether they are driven by a mere increase in ecological opportunity, or if an elevated ‘genomic potential’ is required, often linked to demographic events, hybridization and/or genome evolution. Due to its complex geological history, complemented by strong climatic zonation (humid East versus dry West) and sharp elevation gradients, New Caledonia hosts a rich but threatened biodiversity along its highly heterogeneous soils including ultramafic, serpentines, limestone and sedimentary soils. *Diospyros* (persimmon trees, Ebenaceae) colonized the archipelago four times during the last 20MY, but only the second event gave rise to a radiating clade of ca 30 extant species (including several cryptic species) that at present take advantage of all niches within this biodiversity hotspot, except for mangroves. Previous results suggested that this rapid speciation on New Caledonia has been mainly driven by divergent adaptation to varied edaphic conditions, which happened recurrently during the last million generations. The other three New Caledonian *Diospyros* lineages comprise at most five species, and remain highly restricted in area and types of habitats occupied. We show that, without a change in ploidy, the radiating group has on average twice the genome size and increased TE dynamics compared to non-radiating relatives. The high genome dynamics and TE rich genomes inherently complicate recovery of phylogenetic signals in the radiating clade. In addition, the fast diversification events render reproductive

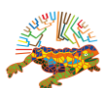
barriers incomplete and frequent incomplete lineage sorting. Using 27 newly assembled genomes and whole genome data for over 500 accessions, we investigate the potential drivers of adaptive variation in this group. After aligning short reads to a newly constructed high-quality reference genome, SNPs were called and filtered to avoid complicating effects of factors such as transposable element expansion and paralogous regions. Thereafter a species phylogeny as well as species networks have been built from “genomic window trees”, ideal for large datasets and clades that display non-tree-like behaviour. Finally elemental compositions of soil and corresponding *Diospyros* plants (leaves were sampled) growing in them were used to determine whether *Diospyros* species from the radiated clade indeed show distinct adaptive traits while also tracking the likely phylogenetic route of their evolution. Our results give evidence for i) pervasive introgression across the history of the radiating group, including of adaptive alleles from related species that share similar environments, in particular those occupying heavy metal rich soils, ii) TE-induced structural variation with potential regulatory effects, iii) environment-specific sorting of ancestral genetic variation, and iv) lineage-specific de novo evolution of alleles. Although the evolution of island biotas is intuitively expected to be mainly shaped by drift rather than natural selection, our results suggest that adaptive processes, often linked to high genome dynamics, introgression and gene family evolution, are playing important roles in the radiation of New Caledonian persimmons.





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S42 - Predicting evolutionary change in ecologically relevant contexts



Antipredator phenotypic plasticity and evolutionary divergence in a freshwater snail

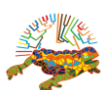
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POSTER SESSION 3 - **P03.192**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

As outlined in the theory of plasticity-led evolution, phenotypic plasticity, which allows individuals to adjust their phenotype in order to optimally respond to their environment, may precede genetic change. This concept is supported by several studies suggesting that phenotypes of derived populations often match the plasticity of a contemporarily existing ancestral population proxy. One of the most convincing examples of adaptive phenotypic plasticity is the induction of antipredator defenses in prey animals. Here, we conduct a long-term experimental evolution study with the freshwater snail *Physella acuta*, a classic model system for studying antipredator responses. Following a split-clutch design, in the first generation, we determined the extent of alarm cue-induced antipredator plasticity in behavior, morphology and life-

history. We also assessed epigenomic and genomic profiles. Subsequently, we raised offspring either in the continuous presence or absence of conspecific alarm cues for multiple generations. Despite the absence of selection in our design, the epigenetic modifications underlying phenotypic change may increase the likelihood of mutations to emerge at the same or neighboring loci. Here, we report first results from this long-term study up to the 6th generation, showing to what extent evolutionary divergence between populations has already emerged on a phenotypic level. We might also be able to report initial results on the corresponding epigenomic and genomic changes to disentangle parental effects from genetic change. Our findings may contribute to a better understanding of plasticity-led evolution.



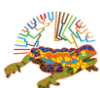
Assessing the genetic vulnerability of *Myotis velifer* to white-nose syndrome

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POSTER SESSION 3 - P03.198, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The emergence of novel diseases can exert immense pressure on wild populations. Such is the case for white-nose syndrome, which has devastated cave-dwelling bat populations since its introduction to North America in the early 2000's. The disease is caused by the fungal pathogen, *Pseudogymnoascus destructans*, which infects bats during hibernation. The Cave Myotis, *Myotis velifer*, was originally believed to be safe from the disease because it inhabits the warmer southern half of North America, with shorter winters. However, as the disease has spread south, records of mortality in this species have been reported. This research comprises an extensive collaboration between US and Mexican states to sample a large proportion of *M. velifer*'s range. We generated ddRadSeq data from 377 individuals, collating a dataset of over 20,000 variant sites. We used a range of techniques in population genomics to examine the population structure across its

range, including exploring associations with ecoregions, and investigated whether the genomic data correlates with the current posited sub-species groupings. We conducted genotype-environment association (GEA) analysis to identify alleles linked to environmental conditions across the range of the bat and disease state of the bats. In combination with information on population structure and connectivity, we used the GEA analyses to infer the adaptive potential of *M. velifer*. Particular focus was placed on sites with links to disease survival, including genes related to immunity, fat storage, and hibernation. This study provides information on the conservation requirements of the species and estimates population vulnerability as white-nose syndrome spreads into the range, thus informing future conservation management. Moreover, we highlight the importance of understanding evolutionary responses to emerging pathogens in wildlife populations.



Breastfeeding difficulties and maternal trade-offs in rural southern Poland

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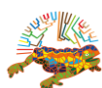
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POSTER SESSION 3 - P03.201, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Exclusive breastfeeding during the first six months of life is assumed to be optimal and universally achievable. Yet, infant feeding practices vary across communities and are shaped by socio-economic and cultural conditions. One of the key reasons for maternal behaviours diverging from medical recommendations is breastfeeding difficulties, which frequently lead to early cessation. Breastfeeding places considerable demands on both maternal energy and time. Previous work in high-income contexts has framed maternal infant feeding strategies as being the result of trade-offs in energetic and time investments, pointing to the utility of Life History Theory (LHT). However, no studies have examined breastfeeding difficulties as a potential consequence of these trade-offs, arising as mothers cope with competing demands and shifting conditions. The likelihood of some problems (mastitis, engorgement, low milk supply) is increased by infrequent feeds, indicating the salience of a LHT perspective. This study explores the prevalence and distribution of breastfeeding difficulties in rural southern Poland, an agricultural area where women have historically returned to farming shortly after birth.

We analysed preliminary data from 62 rural Polish women aged 21-77 (mean = 50.5, SD = 15.65), collected through long-form demographic interviews including detailed questions about infant feeding practices. Data collection of a larger sample (N>500) will be completed in May 2025.

Initial findings reveal that breastfeeding difficulties were common (76%) and occurred across all age cohorts. The most frequently reported issues were low milk supply (47%), mastitis (40%), breast swelling (38%), and sore or cracked nipples (31%). Older women, who were engaged in intensive farming, more often reported perceived milk supply problems, linking them to the demands of agricultural labour. In contrast, younger women more frequently described the negative psychological effects of these difficulties, such as sadness, anxiety, and guilt. Although not breastfeeding is generally denounced in this population, experiencing difficulties tended to mitigate these negative views – with 90% agreeing that ‘there is nothing wrong if a woman does not breastfeed when she has problems with it.’ Historically, intensive farming appears to have necessitated trade-offs in breastfeeding duration, shaped by both maternal choice and energy constraints, which may have contributed to breastfeeding difficulties. Today, with deeper market integration, the infant-feeding landscape has changed. Nevertheless, breastfeeding difficulties, societal pressures, and the realities of returning to work continue to influence maternal decisions. Further studies will explore the influence of age and subsistence on these observed patterns, aiming to better understand the evolving trade-offs in changing rural contexts.



Complex ecological strategies drive genetic distribution of *Arabidopsis thaliana* in natural urban habitats.

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POSTER SESSION 3 - **P03.203**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

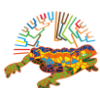
Genetic diversity is shaped by evolutionary forces such as natural selection and drift, influencing organisms' ability to adapt. However, our understanding of the ecological processes that drive genetic adaptation remains limited, hindering efforts to mitigate the effects of climate change. To unravel adaptation mechanisms, it is crucial to monitor adaptation in action within natural habitats. The association between environmental disturbance and ecotypic diversity of *Arabidopsis thaliana* in urban habitats points to local adaptation to environmental heterogeneity. Here, we designed an experiment to dissect the processes driving such pattern in entirely natural conditions.

We generated an F₂ progeny from two locally adapted *A. thaliana* populations in distinct urban habitats in Cologne, Germany. Seeds were sown in 500 natural patches, each characterized by ecological parameters such as disturbance, nutrients, light, and soil composition. We monitored establishment, persistence, and variation in life history traits including population size, flowering time, and seed dormancy. Additionally, seeds

from established populations were grown in common garden to quantify their genetic differentiation during the process.

Established populations differ in life-trait history, displaying a wide range phenotype: short or long life cycle, early or late flowering time and varying dormancy requirements. We reveal the complex millefeuille of interacting ecological filters that shape population diversity. Several abiotic factors consistently affect establishment, while others showed context-dependent effects, from exclusion to facilitation. We further show that the gradient between interspecific competition and environmental disturbance selects for different flowering time alleles. Genetic diversity thus extends habitat suitability.

Taken together, we demonstrate that the city provides an open-air lab for understanding how environmental heterogeneity shapes genetic divergence. I will further present our efforts to uncover genomic footprint of spatially heterogeneous selection in established populations.



Correlated genomic patterns of introgression across space despite contrasting hybridization histories.

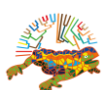
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POSTER SESSION 3 - **P03.207**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Patterns of introgression at genomic resolution have revolutionized our understanding of reproductive isolation and the porosity of species boundaries. Yet, we still know little about the forces that shape these patterns, including how they differ across populations under different ecological, demographic, and reproductive conditions. The genetic mechanisms underlying reproductive isolation have been investigated using both trait mapping approaches and hybrid ancestry patterns, but these methods are rarely compared directly. Through a comparison of geographically disparate contact zones, we investigate the scope of parallelism in patterns of genomic ancestry within a pair of monkeyflower species, *Mimulus guttatus* and *Mimulus nasutus*. In addition, we take advantage of known candidate loci and QTL for premating and postmating reproductive barriers between these species to ask how

well trait mapping approaches predict patterns of ancestry in wild hybridizing populations. We find that the extent and timing of introgression varies considerably across space, at both small and large spatial scales. Parallelism in patterns of genomic ancestry is strongest for geographically proximal groups but remains significant even for populations separated by ~1000km. We find evidence for the selective filtering of introgressed alleles from more admixed to less admixed groups, providing a window into the adaptive introgression process. Finally, we see mixed evidence for correspondence between known reproductive isolation candidate loci and introgression outliers, arguing for the complementarity of different approaches to studying reproductive isolation. Overall, our findings highlight the complexity of factors shaping hybridization patterns.



Cryptic evolution of body size in response to climate change in the Alpine marmot (*Marmota marmota*)

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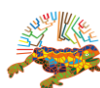
POSTER SESSION 3 - **P03.214**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Understanding the evolutionary processes shaping key life-history traits is crucial for predicting how species cope with rapid environmental changes. Over the last 20 years, advances in quantitative genetics have significantly improved our ability to detect and predict selection responses in natural populations. However, the complexity of natural environmental conditions continues to challenge our understanding of evolutionary trajectories in the wild.

Body size is a fundamental life-history trait, influencing a wide range of biological processes such as fitness. Selection for larger body size is thus the rule in many organisms. Despite this, ongoing declines in body size have been frequently reported across several taxa. So much so that body size shrinkage has been proposed as a third universal response to climate change, alongside geographical shifts and phenological advances. The adaptive significance and underlying mechanisms of body size decline remain poorly understood. Based on 34-year longitudinal monitoring of Alpine marmots (*Marmota marmota*) in the French Alps, we found that body mass of juveniles declined by -17.1g [-21.1; -8.3] per generation. We investigated the temporal trend of this change within a quantitative genetic framework to disentangle evolutionary and plastic responses. We found positive directional selection favoring larger individuals, notably through higher first year survival, as typically observed in mammals. In response, predicted breeding

values from a Bayesian animal model demonstrated an overall genetic change of +6.0g [0.8; 11.0] per generation, as predicted by the Roberston-Price equation, and unlikely to be explained by genetic drift alone. Also, genetic changes were highly non-linear with a pronounced response during the years 1997-2005 (+26.7g [8.1; 44.4] per generation) and a markedly weaker response after 2005 (+2.5 [-4.5; 9.6]).

Altogether, our results reveal a case of cryptic evolution where natural selection slows down the detrimental reduction in body size associated with increasingly unfavorable environmental conditions for marmots. In particular, the decrease of snow cover leads to reduced insulation of burrows and colder temperatures, resulting in poorer conditions of females at breeding time and lighter offspring. Socially mediated effects might also impact such response as significant changes in social structure occurred during the course of the study. The slowing of the evolutionary response in years 2006-2023 remains puzzling as body size is still declining and is smaller than its original average, although additive genetic variance is significantly greater than zero. This appears to result from a decrease in the strength of selection in the more recent period. We investigated how ecological, demographic, and social drivers may affect selection pressure on body size, and highlight implications for predicting evolutionary trajectories under climate change.



Does Mum always know best? Predicting the consequences of rapid host-preference evolution under climate change in Brown Argus butterfly populations

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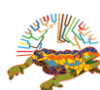
POSTER SESSION 3 - **P03.183**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Ecological communities are responding to climate change through rapid evolutionary shifts. However, even in systems where we have clear genomic evidence of recent adaptation, the ecological and phenotypic consequences of adaptive shifts often remain unexplored. In the UK, the Brown Argus butterfly (*Aricia agestis*) has exhibited a widespread and ongoing northward range expansion in response to climate change. This has been underpinned by an evolutionary shift in maternal host preference, from the chalkland restricted but locally common Rockrose (*Helianthemum nummularium*) to more broadly distributed but locally rare Geraniums (*Geraniaceae*). Such rapid adaptation offers a unique opportunity to test how the novel biotic interactions that need to evolve quickly to allow climate-driven geographical range shifts affect individual fitness and community resilience.

Using common garden assays of host preference on wild-caught females sampled across the UK in 2003, 2013, and 2023, we show for the first time that female preference for Geranium or Rockrose hosts vary among individuals within sites and to different extents at different locations, despite genomic signals of recent selective sweeps on these traits. We also show that host preference shifts are no longer confined to new parts of *Aricia*'s range, with a narrowing of oviposition preference towards Geranium

hosts recorded in long-established sites. To assess the fitness consequences of this host preference evolution, we tested offspring performance on both host plants. We found that irrespective of maternal host plant preference, geranium food plants confer pervasive fitness benefits under laboratory conditions including higher survival, faster development, larger adult body size, and higher mass-independent metabolic rates – all of which are likely to favour population persistence where Geranium hosts are common. This raises the question of why Rockrose preference persists in some females, albeit at much lower frequencies over time.

Despite the apparent advantages of switching to Geranium, these evolutionary shifts to now accessible host plants may not be without their costs. The suitability of geranium as a host is likely to be compromised under future warmer, drier conditions, when compared to Rockrose, which is more resilient to environmental variability, as supported by lab-based experiments and field data on population density shifts in *Aricia* populations across years. Together these findings highlight a central challenge in evolutionary biology – what happens when traits that are adaptive in the short term spread rapidly through populations, but risk becoming maladaptive as environmental conditions continue to change?



Ecological context drives introgression outcomes in experimental *Drosophila* hybridization

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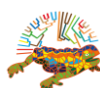
POSTER SESSION 3 - P03.216, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: In rapidly changing environments, predicting evolutionary outcomes requires understanding how ecological context shapes selection pressures. Host-plant chemistry represents a critical but understudied factor influencing evolutionary change in herbivorous insects. Using cactophilic *Drosophila antonietae* and *D. koepferae* - species with divergent host-use strategies - we examine how host chemistry affects developmental stability and phenotypic outcomes in experimental hybrids, providing insights into eco-evolutionary dynamics.

Materials and Methods: We generated F1 hybrids and backcrosses, rearing each lineage on four host cacti media that vary in chemical defenses. To quantify introgression outcomes, we used geometric morphometrics of adult wings, analyzing size, shape, and fluctuating asymmetry as proxies for developmental stability and host-dependent selection. Wing phenotypes were compared to parental controls to identify transgressive traits and host-specific selection pressures.

Results: Host ecology strongly predicted hybrid viability and developmental patterns. Backcrosses to *D. antonietae* failed to develop on the most toxic non-native host, revealing a clear threshold for developmental success. On less toxic hosts, hybrids showed increased developmental instability through elevated fluctuating asymmetry and displayed transgressive wing morphologies, indicating disruption of co-adapted gene networks. These results demonstrate how host chemistry fundamentally alters phenotypic outcomes.

Conclusions: Our study establishes an experimental framework for quantifying host-mediated selection through measurable phenotypic thresholds. By connecting microevolutionary processes with phenotypic outcomes under different ecological conditions, we address critical gaps in predicting evolutionary responses to environmental change. This work advances our ability to forecast population responses to ecological shifts, highlighting the importance of extrinsic factors in shaping evolutionary trajectories - a crucial consideration in our rapidly changing world.



Effect of temperature fluctuations on life history traits and strategies of phytophagous arthropods - a systematic review

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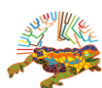
POSTER SESSION 3 - P03.197, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The effect of temperature on the biology of phytophagous arthropods is well understood under constant conditions, but much less is known about how they respond to fluctuating thermal environments. In natural habitats, arthropods are subject to constantly exposed to daily and seasonal variation in temperature. In addition, current global changes are accelerating environmental variability, potentially affecting the life strategies of arthropods.

In this systematic review we aim to investigate the effects of temperature fluctuations on the life history traits and strategies of phytophagous arthropods, a taxonomically diverse group of ecological and economic importance. The fitness of these ectotherms is directly affected by ambient temperature and may also be indirectly affected by temperature-induced changes in host plant quality. A comprehensive literature search was conducted using the Scopus and Web of Science databases. From the selected articles, we extracted information on the aim of the study, study subject, and the experimental design. We then carried out a critical analysis of the methods used to assess temperature effects, with a particular emphasis on fluctuating thermal regimes. We also collected information on the life history traits and strategies studied.

The results indicate that current knowledge of the effects of temperature fluctuations on phytophagous arthropods remains limited and highlights significant methodological challenges. Preliminary findings suggest that compared to constant conditions, fluctuating temperatures tend to accelerate development, increase hatching rates, improve survival, fecundity, and growth, although often at the expense of reduced body mass. However, the observed effects appear to depend on the magnitude and range of temperature variation: fluctuations within optimal ranges generally improve performance, whereas exposure to thermal extremes produces mixed results, either alleviating physiological stress or causing cumulative damage. Much of the existing research has focused on the impact of climate change on agricultural pests, with strong bias towards Lepidoptera and a limited focus on mites, highlighting a notable research gap. It is important to understand how temperature changes affect phytophagous arthropods in order to predict their distribution patterns, anticipate population outbreaks, and develop effective pest management strategies under changing environmental conditions.

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Escaping from a stressor: evolution of larval development under nutritional shortage

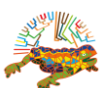
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POSTER SESSION 3 - **P03.180**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

In nature, organisms often face periods of food shortage, which can severely impact metabolism, fitness, and survival. Immature stages, like juvenile insects, are especially vulnerable due to limited mobility, restricting their ability to reach new nutrient sources and most of the time they have no other option but to continue development. Therefore, natural selection is expected to favor physiological, behavioral and life history adaptations that alleviate the negative consequences of nutrient scarcity for Darwinian fitness. In our lab, *Drosophila melanogaster* larvae evolved on a diet with only 25% of the standard nutrient content over 350 generations. As a result of this experimental evolution, these populations are now able to develop much faster in an undernutrition context than Control

populations evolved on standard diet and only emerge at a slightly smaller adult body weight. Such changes can be accomplished by faster growth throughout development but also by shortening the larval wandering period and other potential changes in relative duration of different larval stages. Here I will report on a study in which I characterized in detail the differences in growth (in terms of protein accumulation), accumulation of fat reserves and the timing and size at which Selected larvae achieve a growth advantage over Controls under nutrient-poor conditions. The project contributes to the understanding of mechanisms mediating the evolution of enhanced tolerance to juvenile organisms forced to develop despite nutritional constraints.



Evolution of the seasonal preference of pathogens

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POSTER SESSION 3 - P03.210, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Pathogens often exhibit seasonal epidemics. Influenza is prevalent in winter, while enteroviruses are prevalent in summer. How have such pathogen epidemic calendars evolved? Here, we study the evolution of seasonal preferences of pathogens in periodic environments. Assuming that the infectivity of pathogens is determined by the seasonal preference of pathogens for periodic external environmental fluctuations (temperature, humidity, host activity, etc.) and the pathogen's seasonal preference traits, we investigate the evolutionary dynamics of the preferred season using an adaptive dynamics approach. We found that the evolutionary dynamics vary significantly depending on the magnitude of environmental fluctuations. When

environmental fluctuations are large, pathogens' seasonal preference converges on a specific season (ESS preference), while in cases of small environmental fluctuations, the preferred season shifts earlier over time (evolutionary drift). Additionally, by altering the range of preferred seasons, more complex dynamics such as the maintenance of seasonal preference polymorphism and polymorphic evolutionary drift can be observed. Our findings may help explain seasonal preference polymorphism in parainfluenza viruses and some enteroviruses and contribute to a deeper understanding of the evolution of life history traits in pathogens.



Evolution to salinity in communities

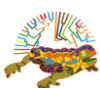
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POSTER SESSION 3 - P03.189, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Due to global change and anthropogenic activities, freshwaters are becoming more saline. Increases in salinity can affect species and entire communities living in these freshwater systems via shifts in density and trait dynamics. However, species' populations may not only respond to changes in the abiotic environment, but also to density changes of member species in the community. Moreover, previous research has suggested that species may evolve differently to abiotic change when embedded within a community. However, experimental tests of this remain scarce. We used the freshwater ciliate *Colpidium striatum* to study the evolution to increased salinity when populations of the species

evolved alone or embedded within a community. We conducted three common garden experiments after two, four, and five months during a long-term community selection experiment in which single-species and 4-species communities were exposed to no, slow or fast increasing levels of salinity. In the common garden experiments, we exposed isolated single species populations to no or high salt concentrations, testing if previous exposures to salt and/or competitive interactions altered their evolutionary response to salt. We found that both the level of environmental change, as well as the community background were able to alter population traits.



Genomic and physiological mechanisms underpinning seasonal adaptation in the copepod *Eurytemora affinis*

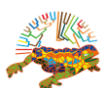
Nascimento Schulze J¹, Hahn A¹, Clemmesen C¹, Avgerinou G¹, Stumpp M², Brennan R¹

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POSTER SESSION 3 - P03.185, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Species with short lifespans experience environmental heterogeneity, both spatially and temporarily, including seasonal changes that occur across generations. Balancing selection, a process that maintains genetic diversity within populations by shifting allele frequencies alongside environmental changes, may facilitate rapid adaptation to fluctuating environments. Identifying the genomic mechanisms driving fast adaptive responses, and how these shape physiological processes, can improve our ability to predict whether and how taxa may adapt to human-driven climate change. In this study, we leverage a wild population of the copepod *Eurytemora affinis* from the Baltic Sea (Kiel, Germany) to identify the genomic and physiological responses to rapid environmental change. Whole-genome sequencing of pooled individuals collected over a 13-year period (2009, 2011, 2015, 2022) in both spring and summer, was used to identify rapid adaptation to seasonal

change. We found nearly 1,000 loci consistently responding to seasonal variation across all years, indicating ongoing rapid evolution via balancing selection. To identify the phenotypic basis of adaptation, copepods were collected at four timepoints from spring to summer of 2024 and common-gardened for three generations to two experimental temperature regimes simulating the local seasonal sea surface conditions (7°C and 16°C). We measured key fitness traits: growth, fecundity, and metabolic and feeding rates. Cold-adapted populations generally had lower energy requirements and outperformed warm-adapted populations in growth and fecundity, highlighting potential trade-offs in adaptation to fluctuating environments. These findings provide genomic and phenotypic evidence of rapid evolutionary responses to seasonal change in the copepod *Eurytemora affinis*.



Genomic Insights into Kaimanawa Horses: Demography, Diversity, and Conservation Strategies for a Feral Population

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POSTER SESSION 3 - P03.195, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Domesticated horses (*Equus ferus caballus*) played an important role in shaping human society. By revolutionizing transportation, agriculture, and warfare, the lives of humans and horses became intensively intertwined. However, during modernity, humans started intensive selective breeding programs. This not only resulted in upgrades in performance and usability, but also in a loss of around 16 % genetic diversity compared to their already extinct wild ancestors. To maintain their genetic diversity in the future, it has become increasingly urgent to find genetically diverse populations for future breeding programs and conservation.

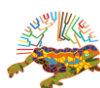
Feral horse populations, like the Kaimanawa horses in New Zealand, bear the opportunity to still contain some rare variations that have been lost in modern breeds. Introduced by early European settlers in 1815, these horses underwent dramatic demographic changes, including bottlenecks and population fluctuations and now thrive under governmental protection. Their well-recorded demographic history, provides a rare and valuable opportunity to explore population dynamics and assess the effectiveness of various conservation strategies.

Materials and methods: Private owners of tamed feral Kaimanawa horses provided hair samples upon request. We randomly selected and genotyped 142 of these using the 80K GGP Equine v4 SNP array. For comparative analysis, 23 other horse breeds were included, resulting in a final data set of 646 individuals and ~18K SNPs. To gain deeper insights into the ancestral proportions shared between the included breeds and the Kaimanawa horses, an admixture analysis was conducted. Additionally, Principal Component Analysis (PCA) was performed to detect genetic similarities between the Kaimanawa horses and breeds with known genetic contributions. Genetic diversity parameters—such as observed homozygosity, inbreeding coefficient (*F*), and nucleotide diversity—were also assessed. Genome-wide nucleotide diversity, calculated

using only the Kaimanawa horse samples (142 individuals and 55,423 SNPs), was used to detect potential genomic regions under selection.

Results: 36.2 % of Kaimanawa horses were found to share second-degree or closer genetic relationship (Kinship > 0.0884), highlighting a high degree of relatedness within the population. Admixture analyses revealed genetic contributions from several domestic breeds, including Arabian, Clydesdale, Shetland Pony, Standardbred, and Thoroughbred horses, and revealed the existence of at least two subpopulations within the Kaimanawa group. These findings were further supported by PCA and PCAir analyses, which also indicated genetic similarities between Kaimanawa horses, Welsh Ponies and French Montagne horses. Compared to the other 23 horse breeds—ranging from a minimum observed homozygosity of 0.658 in Hannoverians to a maximum of 0.761 in Exmoor Ponies—the Kaimanawa horses exhibited the sixth highest value at 0.719, suggesting reduced genetic diversity. Additionally, genome-wide scans for nucleotide diversity identified specific regions that may be under selection, highlighting the genomic impact of demographic history and feral adaptation.

Conclusions: The high relatedness observed within the Kaimanawa horse population might be the result of recent bottlenecks and consequently small effective population size. Genomic analyses reveal a complex ancestry with contributions from multiple domestic breeds, as well as signatures of selection which may represent unique adaptations to their feral life. To preserve the existing genetic diversity and support the long-term viability, management strategies should account for the observed subpopulation structure. This study provides a scientific foundation for future management efforts—aimed not only at protecting the genetic diversity of the Kaimanawa horses, but also underscores the importance of applying genomic insights to the conservation of other feral horse populations worldwide.



Host shift changes population dynamics and phenotypic plasticity in seed beetles

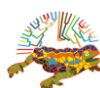
Savković U¹, Budečević S¹, Vlajnić L², Đorđević M¹

¹Department of Evolutionary Biology, Institute for Biological Research “Siniša Stanković” – National Institute of the Republic of Serbia, University of Belgrade, ²Institute of Zoology, Faculty of Biology, University of Belgrade

POSTER SESSION 3 - P03.193, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The ability of phytophagous insects to expand their diet and find new food sources is one of the most important aspects of insect adaptation to novel environments. Changes in diet can trigger eco-evolutionary dynamics in insect populations and significantly alter insect behaviour, food digestion and physiology, and ultimately how changes in life history strategies relate to population dynamics. One of the most important mechanisms in the response of populations to a changing environment is undoubtedly phenotypic plasticity. Phenotypic plasticity can facilitate or hinder the process of adaptation to a new environmental factor. If adaptive, phenotypic plasticity could help populations establish a functional phenotype specific to the environment to which they are exposed. The ubiquity of phenotypic plasticity can help us understand how organisms perceive, process and respond to changes in the environment, and it can inform us about the short-term (ecological) and long-term (evolutionary) consequences of such changes. Here we used populations of seed beetles (*Acanthoscelides obtectus*) reared over 150 generations on three different host plants (common beans, chickpeas and mung beans). We performed a reciprocal transplant experiment with iso-female lines to test the level of phenotypic plasticity of life

history traits. In addition, we used life history datasets to construct the life tables and applied a jackknife method to calculate population parameters to observe population dynamics after a single-generation exposure to a new plant host or after a transgenerational exposure. This approach allowed us to observe jackknife variances, confidence intervals and pairwise comparisons between experimental groups for population parameters. We found different life history strategies, different environmental sensitivities and population dynamics for each seed beetle population. Populations reared on common beans demonstrated stable preadult development on all three host plants and high sensitivity in reproductive performance. Populations reared on chickpea were highly specialized for development on chickpea, while their reproductive performance was high regardless of the host plant. Populations from mung bean showed a higher degree of variance and plasticity in both preadult (larval survival) and adult (fecundity) traits. This work is discussed in the context of how the diet of insects (i.e. different plant hosts) can affect population dynamics and how long-term exposure to a particular environmental condition can influence the ecological competence of organisms.



'Inter-island genetic connectivity and selection in the Avian Vampire Fly across the Galápagos archipelago'

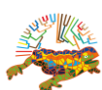
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POSTER SESSION 3 - **P03.186**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Islands serve as model systems for understanding the repeatability of evolution during biological invasions, whereby invaders may undergo multiple colonisations across an archipelago. To establish in a new environment, invasive parasites must overcome evolutionary challenges that can determine host-pathogen dynamics and impact life-history traits, behaviour and morphology. The avian vampire fly (*Philornis downsi*) is an invasive ectoparasite first recorded in nests of Galápagos birds in 1997. This high-risk ectoparasite causes substantial mortality in Darwin's finches, feeding on the blood and tissue of developing nestlings. Since its discovery in Galápagos, changes in host-parasite dynamics have been recorded, including shifts in oviposition behaviour and a reduction in female abdomen size, indicating 'downward fecundity selection' over time. The aims of this study are to 1) detect if there are replicate selection signatures in avian vampire fly populations across the Galápagos islands, and 2) determine if candidate genes under selection that associate with morphology show island-specific patterns, and thus, different trajectories of fecundity selection. To explore this, we used low-coverage whole genome

sequencing for 189 avian vampire flies collected across five of the major Galápagos islands. We first consolidate patterns of genetic structure and migration across islands. We then investigate signatures of phenotypic selection across islands by integrating a high-resolution morphological dataset. Selection scans and genotype-phenotype analyses will be explored to identify island-specific candidate loci under selection in relation to island and morphological traits. Functional gene annotation will reveal the biological significance of candidate genes. By elucidating patterns of gene flow and selection in invasive parasites within a replicate island system, we can better understand how replicate patterns of local adaptation can emerge. It is predicted that the avian vampire fly may display variation in selection signatures across islands of the Galápagos due to island differences in environmental conditions, host resource availability and human impacts. This study offers a deeper understanding of the evolutionary mechanisms that enable invasive species to move and adapt across novel replicated environments, in the context of host-parasite interactions.



Is Bergmann's rule valid for terrestrial vertebrates?

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POSTER SESSION 3 - **P03.181**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Bergmann's rule posits that animals tend to have larger body sizes at higher latitudes with colder climates, but its applicability across vertebrate groups has been a subject of debate. While earlier studies suggested that the rule predominantly applies to endotherms, this hypothesis remains contested due to inconsistent empirical support across taxonomic groups and levels. Understanding the generality and mechanisms of Bergmann's rule is critical for elucidating how species adapt to environmental gradients and respond to climate change. In this study, we examined species- and population-level data across four major terrestrial vertebrate taxa (amphibians, reptiles, birds, and mammals) to assess the strength and consistency of Bergmann's rule.

Using Bayesian phylogenetic generalized linear mixed models and meta-analysis, we analysed body size–latitude correlations at both species and population levels. We also evaluated the influence of ecological and life-history traits (e.g., habitat, diet, activity patterns, migration). Our dataset included over 30,000 for species level and 786 population level, representing a comprehensive assessment of latitudinal body size trends across terrestrial vertebrates.

We found that Bergmann's rule applies to endotherms (birds and mammals), as evidenced by a positive correlation between body size and latitude at the species level. In contrast, the rule does not hold for

ectotherms (amphibians and reptiles), which showed no significant or reversed trends. At the order/family level, support for Bergmann's rule was slightly higher in endotherms (34%) compared to ectotherms (22%). Similarly, at the species level, endotherms exhibited greater adherence to the rule (22%) than ectotherms (18%). However, contrary to expectations, Bergmann's rule was not stronger at the population level (21% of species) than at the species level (24% of families or orders). These findings highlight the context-dependent nature of Bergmann's rule, with thermoregulatory mechanisms, ecological and life-history traits key roles in shaping latitudinal body size patterns.

Our study demonstrates that Bergmann's rule is not a universal principle but rather a context-dependent pattern influenced by thermoregulatory mechanisms, ecological traits, and phylogenetic history. While endotherms show stronger adherence to the rule, ectotherms exhibit highly variable or reversed trends, reflecting their differential sensitivity to environmental changes. These findings underscore the need for a more nuanced understanding of Bergmann's rule across taxa and highlight the vulnerability of ectotherms to climate change due to their limited adaptive capacity. Future research should focus on disentangling the ecological and evolutionary drivers of body size variation to better predict species responses to global environmental changes.



Life-history trade-offs explain local adaptation in *Arabidopsis thaliana*

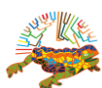
Brachi B^{1,2}, Filiault D³, Pisupati R³, Dahan-Meir T³, Igolkina A³, Anastasio A¹, Box M⁴, Duncan S⁴, Karasov T¹, Kerdaffrec E³, Merwin L¹, Morton T¹, Nizhynska V³, Yu.-Novikova P³, Rabanal F³, Tsuchimatsu T³, Säll T⁵, Dean C⁴, Holm S⁶, Bergelson J^{1,7}, Nordborg M³

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POSTER SESSION 3 - P03.200, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Local adaptation has been demonstrated in many organisms, but the traits involved, and the temporal and spatial scales at which selection acts are generally unknown. We carried out a multi-year study of 200 accessions (natural inbred lines) of Swedish *Arabidopsis thaliana* using local field sites and a combination of common-garden experiments that measured adult survival and fecundity, and selection experiments that measured fitness over the full life cycle. We found evidence of strong and variable selection, with particular genotypes favored more than five-fold in certain years and locations. Fecundity showed evidence of classical local adaptation, with accessions generally performing better close to their home. However, southern accessions

usually had the highest fecundity--but were far more sensitive to harsh winters and slug herbivory, which strongly decreased both survival and fecundity. Accessions originally sampled on beaches had low fecundity in all environments, but massively outperformed all other accessions in the selection experiments, presumably due to an advantage during seedling establishment associated with their very large seeds. We conclude that local adaptation in *A. thaliana* reflects strong temporally and spatially varying selection on multiple traits, generally involving trade-offs and different life-history strategies, making fitness difficult to predict and measure.



Long-term evolution experiments fully reveal the potential for thermal adaptation

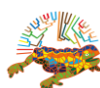
Antunes M¹, **Grandela A**¹, Matos M¹, Simões P¹

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POSTER SESSION 3 - P03.190, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Evolutionary responses may be key in allowing organisms to cope with the sustained effects of climate change. However, a clear understanding of the dynamics of adaptation to warming environments is still lacking. Addressing how reproductive success evolves in such deteriorating environments is extremely relevant, since fertility is constrained at lower temperatures than survival. Experimental evolution under a warming environment can elucidate the potential of populations to respond to rapid environmental changes. The few studies following such framework lack analysis of long-term response. Here, we focus on the long-term thermal evolution of two *Drosophila subobscura* populations from different European latitudes under warming temperatures. We tested the reproductive success of these populations in the ancestral (control) and warming environment after more than 50 generations of thermal evolution. We found a general

adaptive response to warming temperatures in the long term, since populations evolving in the warming environment showed increased performance in that environment relative to the respective control populations. On the other hand, no clear response was observed in the ancestral environment. Coupled with data from previous generations, we highlight a slow pace of adaptive response and differences in that response between populations of distinct geographical locations. These findings demonstrate the need for long-term evolution experiments to fully reveal the potential for thermal adaptation. It also highlights that the scrutiny of different populations is needed to better understand the variation in evolutionary responses within a species. Accounting for these sources of variation - both temporal and spatial - will allow for more robust assessments of climate change evolutionary responses.



Predators in the eco-evolutionary dynamics of arthropod communities

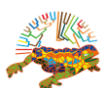
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POSTER SESSION 3 - P03.206, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Predation plays a key role in shaping ecological communities and their dynamics. Predators can affect prey communities in two ways – through consumptive effects (CEs) which are lethal, or non-consumptive effects (NCEs) such as fear-induced dispersal. While CEs have been broadly integrated into an eco-evolutionary framework, the role of NCEs in this context remains less understood. Past work showed that variation in local adaptation of *Timema cristinae* stick-insect camouflage influences arthropod communities by affecting bird

predation, and that these effects depend on palatability and dispersal ability of prey species. We conducted a paired experiment in natural conditions using *T. cristinae* stick-insects and coexisting arthropods to investigate the effects of fear on the community. We specifically measured the impact of perceived predation risk in triggering immediate arthropod dispersal. Our results shed light on the importance of both fear and consumption in eco-evolutionary dynamics.



Predictability in coevolution under hierarchical competition

Fragata I¹, Godinho D², R Rodrigues L¹, Cruz M¹, Costa-Pereira R³, Godoy O⁴, Magalhães S¹

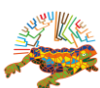
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POSTER SESSION 3 - P03.212, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Understanding how interactions between species shape adaptation to new environments improves our ability to predict community dynamics in a changing world. While it is known that biotic and abiotic stresses jointly shape evolutionary dynamics of interacting species, the extent to which coevolution with competitors contributes to their adaptation to a novel environment remains poorly understood.

Here, we tackled this question by performing experimental (co)evolution of the spider mite crop pests *Tetranychus urticae* and *T. evansi* competing in an abiotically heterogeneous environment composed of tomato plants exposed to different cadmium concentrations. We established five selection regimes, each replicated five times: two in which each species evolved in the absence of competitors; two in which each species evolved in the presence of its interspecific competitor that itself did not evolve, and one in which both species coevolved. We then quantified changes in a suite of traits linked to (1) adaptation to the new environment (fertility, viability and intrinsic growth rate), (2) competitive

interactions (intraspecific and interspecific competitive ability and growth rate in the presence of competitors) and (3) habitat choice (host plant preference). We found that *T. evansi* (the stronger competitor) exposed to both evolving and non-evolving competitors improved its performance in the non-cadmium environment. In addition, we found increased sensitivity to intraspecific competition in the selection regimes in which *T. evansi* evolved or coevolved with its competitor. whereas increase in *T. evansi* sensitivity to interspecific competition was only observed in the coevolved regime. In contrast, no changes in performance or sensitivity to competition in *T. urticae* were found across any environment or selection regime. Finally, evolution or coevolution did not affect host preference. Overall, we observed more pronounced evolutionary changes in the stronger competitor, and in most cases with a similar magnitude and direction in both the coevolution and evolution in the presence of a naïve competitor regime. These findings suggest that the outcomes of these two processes may not differ and be highly predictable.



Predicting community response to warming and heat waves using thermal performance of component species

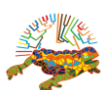
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POSTER SESSION 3 - **P03.211**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Temperature plays a critical role in shaping the physiology and interactions of ectothermic organisms, making it a key factor in understanding community responses to climate change. In this study, we investigate the thermal performance curves (TPCs) of 11 *Drosophila* species and 8 parasitoid species, using *Drosophila melanogaster* as a common host across all parasitoid treatments. Additionally, we explore a subset of 3×3 host-parasitoid interactions to evaluate how temperature influences interspecific dynamics within a community context. We quantified key fitness-related traits—survival, maturation time, fertility, and starvation resistance—across a temperature gradient ranging from 15°C to 33°C. These traits served as proxies for overall fitness and were used to construct species-specific TPCs. Our results reveal that parasitoid viability declines significantly above 27°C. In contrast, *Drosophila* species exhibit variable survival even at 32°C. However, despite this apparent thermal tolerance, their fertility declines markedly beyond 27°C, indicating reproductive constraints that may limit long-term

population persistence. This difference in thermal tolerance points to a thermal mismatch between *Drosophila* and parasitoids, which could disrupt host-parasitoid interactions under projected climate warming scenarios. The consequences of this mismatch are likely to cascade through ecological networks, potentially altering species abundances, shifting interaction strengths, and destabilizing community structures. By incorporating empirically derived TPCs into ecological models, we aim to predict community responses to temperature fluctuations, offering insights into species adaptation and ecosystem resilience to climate change. This approach allows us to move beyond single-species assessments and instead capture the interactive effects of temperature on ecological communities. This study highlights the complexity of predicting ecological responses and the need for comprehensive models that account for species interactions and thermal sensitivities.



Rapid evolution reveals trade-offs across ecological interactions in a host-parasitoid system

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POSTER SESSION 1 - **P01.024**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Species exist within a dynamic network of ecological interactions, and rapid evolution can modulate these interactions across surprisingly short timescales. While trade-offs between competitive ability and parasitoid resistance are well documented, how such responses generalize across multiple biotic challenges remains less understood. Here, we explore the emergence of evolutionary trade-offs in a controlled laboratory evolution experiment with *Drosophila melanogaster*, focusing on two axes of selection: larval competition and parasitism by *Leptopilina boulardi*.

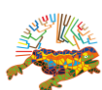
We experimentally evolved a genetically diverse population of *D. melanogaster* for over 15 generations under three regimes: control conditions, high larval competition, and parasitism by *L. boulardi* (Congo strain). After two generations of common garden rearing, we assayed fitness under standardized environments using a panel of parasitoids varying in relatedness and infection strategy: a conspecific strain of *L. boulardi* (France), *L. victoriae*, the generalist *L. heterotoma*, and *Ganaspis xanthopoda*. Selection for competitive ability reduced parasitoid resistance across multiple species, even before gains in competition emerged. Conversely, populations selected for parasitoid resistance do not show improvements or reductions in larval competitive ability, suggesting an asymmetric trade-off dynamic. Furthermore,

evolved resistance to *L. boulardi* (Congo strain) confers increased resistance to its conspecific strain but reduces resistance to *L. heterotoma*, highlighting that cross-resistance effects are strongly contingent on parasitoid identity.

Our findings provide strong evidence for context-dependent trade-offs across species interactions, emphasizing that selection in one domain (competitive ability) can constrain adaptive responses in another (immunity). This highlights the potential for evolutionary constraints to act as stabilizing forces in ecological communities by maintaining phenotypic variation.

By using genetically diverse and large populations, and experimental evolution rather than artificial selection, our study better captures the ecological realism of natural selection acting on whole-organism fitness. This design enables us to infer how hosts might respond in the wild to fluctuating pressures such as crowding and parasitism, and how these responses could shape host-parasitoid population dynamics.

To sum up, we show that evolution in response to one type of interaction can reverberate across a network of others, shaping the trajectory of both resistance and susceptibility in a host population. These findings underscore the need to consider multidimensional selection when predicting host-pathogen coevolution and species coexistence.



Repeatability of evolution in interacting species

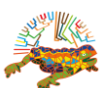
Hablützel L^{1,2}, Bank C^{1,2}

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POSTER SESSION 3 - P03.213, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

A central question in evolutionary biology is the repeatability of evolution - how likely is it that the same evolutionary path is taken given the same initial conditions? And how does the repeatability of evolution differ between environments? Here, we examine how repeatability is affected when species evolve independently versus when they co-evolve with an interacting species. To explore this, we take a theoretical approach by simulating two species navigating interdependent (deformable) fitness landscapes. On these deformable landscapes, the genotypes of both species are mapped to different fitness values, since here, fitness not only depends on the genotype of the focal species but also on the genotypes of the interacting species. Thus, fitness differs between combinations of genotypes, if the fitness landscapes are interdependent. We analyse evolutionary trajectories resulting from a strong selection,

weak mutation (SSWM) regime, focusing on contingencies - critical points where evolutionary outcomes diverge. We identify the frequency of branching points or bottlenecks during the species' adaptive walk on the deformable versus a static fitness landscape. We distinguish between within-species contingencies, where certain genotypes are necessary to reach specific fitness peaks (e.g., due to epistasis), and between-species contingencies, where the presence of specific genotypes in one species enables fitness gains in the other (e.g., due to intergenomic epistasis). This way, we can directly contrast the effects of epistasis within and between genomes. This approach sheds light on how species interactions influence evolutionary dynamics, potentially constraining or expanding the accessible genotype space during adaptation, impacting the repeatability of evolution.



Spatial patterns of intraspecific genetic diversity in herptiles: a machine learning approach to macrogenetics

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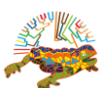
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POSTER SESSION 3 - P03.188, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Intraspecific genetic diversity (ISD) underpins key eco-evolutionary processes, yet its spatial distribution across species' ranges remains poorly characterized at broad scales. Here, we investigate how ISD varies in relation to both geographic and climatic centroids using data from 432 herptile species (244 reptiles and 188 amphibians) across six global regions. For species with at least five georeferenced DNA sequences from different locations, we generated spatially explicit ISD surfaces by interpolating gene-specific data. We then quantified the relationship between ISD and the distance to both geographic and climatic centroids using species-specific Spearman's ρ coefficients. Combining sequence alignments, species distribution models, and comparative analyses, we tested whether populations closer to current niche optima exhibit higher ISD, potentially reflecting greater environmental stability and reproductive success. To further investigate

the factors shaping these patterns, we applied a random forest framework integrating ecological, historical, and methodological predictors, including niche hypervolume, climate stability, range size and sampling effort. This comparative approach allowed us to assess whether ISD is typically concentrated near range centers or more evenly distributed, and to identify traits most strongly associated with these trends. Notably, the strength and direction of ISD-centroid correlations varied among species. Our findings offer new insights into the macroecological drivers of ISD and underscore the importance of integrating molecular, ecological, and historical data to understand spatial patterns of genetic diversity. This work contributes to identifying key populations and regions for conservation and advances our understanding of how evolutionary processes shape genetic diversity across space.



Statistical models of eco-evolutionary dynamics to understand the past and predict future states of ecological communities

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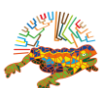
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POSTER SESSION 3 - P03.209, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Biodiversity is typically influenced by numerous environmental, spatial, and stochastic factors, and all of these may impact how communities respond to environmental change. At the same time, populations with genetic variation can also evolve and potentially adapt to new conditions. Researchers who study the impacts of multiple environmental drivers of biodiversity thus have many complex, interacting processes to consider that may shape future community dynamics. How can we distinguish the relative role of different ecological and evolutionary processes in driving species responses to a changing environment, using data typically collected in ecological surveys? Can we use this understanding to forecast how communities will change under future scenarios?

I will present two types of statistical modelling approaches (Approximate Bayesian computation, ABC, and Joint Species Distribution Models, JSDM) that can

help to answer these questions. The methods can distinguish among alternative hypotheses for the eco-evolutionary processes that might impact observed (and future) patterns of biodiversity. The ABC approach can assign probabilities that observed data was produced by alternative models without and with trait evolution occurring on the same time scale as ecological processes, or without and with eco-evolutionary feedbacks, which is useful to assess the impact of evolution for community dynamics. ABC can also estimate model parameters that can be used to predict future community dynamics. The JSDM approach can be modified to include evolving trait data for analysis of community composition change, and I show an application to simulated multi-species experimental data. Both approaches can be used to estimate the relative importance of evolution for community change, and to model changes in species composition in new locations or future points in time.



The response of the life history traits of *Daphnia magna* to the deteriorating and fluctuating temperatures

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POSTER SESSION 3 - **P03.205**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Adverse anthropogenic impacts on the Biosphere have produced environmental change at a rate unprecedented in the Cenozoic and resulted in the shifting of environmental conditions outside of the physiological tolerance range of many species. The two most alarming consequences of global change are the continuous rise of temperature from the pre-industrial era and a detectable increase in the intensity of thermal fluctuations. Predicting the patterns of species' response to climate change requires tracking the variation in fitness-related traits (i.e. life histories) during exposure to thermally changing environments, because these traits may strongly correlate with the probability of survival.

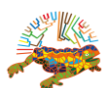
A growing body of experimental studies demonstrated that an increase of both the mean and variation of temperature significantly influences the response of several life history traits, namely reduction of development time and body size at maturation. However, there are still significant gaps in our understanding of how species respond to temperature change, particularly because most research has focused only on abrupt changes to thermal conditions (experimental conditions with fixed temperature differences). To incorporate ecological realism into our studies, we need experiments investigating the effects of gradually changing thermal environments over longer time scales, because the population genetics of adaptation to stressful environments is strongly influenced by the rate of

environmental change (hence, the responses of natural populations may differ under the gradually changing thermal environments). Moreover, we have little information about the relative contribution of phenotypic plasticity (phenotypic change of a character without an underlying genetic change) and genetic adaptations underlying the response of life histories to the changing thermal regimes, which are often difficult to disentangle.

To investigate evolution under fluctuating and deteriorating thermal environments, respectively, we carry out the two experiments with a plankton crustacean, *Daphnia magna*. We subjected the clonal populations of *D. magna* to two different thermal conditions: 1) a regime where temperatures fluctuate in regular intervals and 2) a regime with continuous increase of temperature in regular intervals. To provide insight into the underlying mechanisms of the change of the life history traits, the experimental populations are monitored in a series of reciprocal transplant experiments by measuring five life history traits (span between clutches, body size at birth, somatic growth rate, body size at maturation and life span).

After 30 generations of selection, we detected a significant clone-by-treatment interaction underlying the response of most life history traits monitored. Our results indicate significant genetic basis as potential for the adaptation to the global warming related changes of thermal regimes.

Keywords: *Daphnia magna*, Deteriorating temperature, life history traits



Understanding the Ecology of Sexual Size Dimorphism Through Resource Competition in a Sex- and Size-structured Model

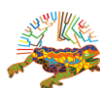
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POSTER SESSION 3 - P03.208, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The variation in morphology between the two sexes of a given species is an important aspect of intraspecific diversity. In particular, sexual difference in body size (sexual size dimorphism, SSD) is a common form of sexual dimorphism. Since the sexes often have the same size at birth, SSD emerges during the development; therefore, it is closely linked to resource competition, sex-specific energetic requirements and investment in reproduction, and size-specific mortality rate. Yet, the ecology of SSD has often been neglected in the past. Emerging evidence showed that ecological conditions can affect the evolution of SSD, whereas the evolutionary changes in SSD also likely impact the ecological dynamics of the given species. Additionally, most models of population dynamics tend to implicitly or explicitly focus on female density only and do not take ontogenetic development into account, limiting their ability to accurately predict the dynamics of sexually dimorphic

species. By explicitly incorporating sexes and SSD in a size-structured population model with an explicit resource, we address how does resource competition between the sexes and different life stages influence population dynamics and persistence, and how this is affected by the direction and magnitude of SSD. In this model, reproductive output depends on resource availability, the adult sex ratio, and sexual dimorphism which emerges from differences in sex-specific energy allocation. We demonstrate how population persistence and adult sex ratio change with varying degrees of SSD and the productivity of the resource. Overall, our study contributes to the existing knowledge on SSD by adding an ecological perspective. Finally, the insights from this modelling study provide the start of a project in which I will investigate different aspects of the eco-evolutionary dynamics of SSD, for instance, the evolution of niche separation between sexes.



Assessing selection on reproductive phenology in forest and urban great tits: including multiple broods matters

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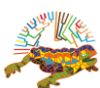
MEETING ROOM 114, August 21, 2025, 10:30 AM - 12:30 PM

Urban areas provide a unique opportunity to study organisms' phenotypic responses to novel human-induced environmental conditions. Many studies have described phenotypic differences between urban and rural bird populations, e.g., in morphology or life history traits. Recently, several studies documented divergences in reproductive phenology and in the associated selection pressures between habitats. However, these studies only focused on first clutches, without considering multiple clutches.

This study investigated the relation between reproductive performance and egg laying date in urban and forest great tits exploiting a 13- year data set. We first estimated and compared laying date between forest and urban habitats. Then, we quantified linear reproductive selection (using number of fledglings as a proxy of reproductive output) on laying date for first clutches in both habitats. In addition, we studied the selective impact of laying date of first clutches on the reproductive outcome per season (fledglings from first, replacement and second clutches combined).

We confirmed that urban birds bred on average earlier compared to their forest counterparts. In first clutches, forest great tits tended to exhibit negative selection favoring earlier breeding whereas statistically non-significant positive selection was found in the city. As concerns the reproductive outcome per season, selection patterns changed as both urban and forest great tits exhibited negative selection favoring earlier first breeding attempt.

Our findings suggest that earlier breeding phenology in urban and forest environments is adaptive because it increases the probability to rear a successful replacement or second brood. Our findings also underscore the need to account for multiple broods when assessing how selection acts on reproductive timing across contrasting environments. A crucial perspective in understanding these dynamics lies in examining how urban environmental conditions, particularly the spatio-temporal variation in food resources, shape reproductive timing and success in urban bird populations.



Conflicting temporal dynamics of selection, additive genetic variance and plasticity in early-life seasonal migration versus residence constrain the potential for rapid adaptive evolution

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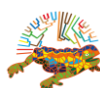
Eco-evolutionary responses to rapid environmental changes will depend on interacting magnitudes of selection, additive genetic variances and plasticity in key phenotypic traits that facilitate population persistence. To generate rapid adaptive evolution of such traits, strong selection must act on heritable trait variation. Yet, in nature, genetic variances and selection may depend on environmental conditions that vary at fine within-year timescales. Any temporal asynchrony of selection episodes and expression of genetic variance could then constrain evolutionary change below initial predictions. Furthermore, the potential to maintain and/or evolve plasticity in critical traits will depend on the dynamics of selection on plasticity expressed in varying environments. Here, plastic responses can cause immediate fitness costs, but selection may ultimately favour expression of plasticity over canalisation at longer temporal scales of environmental variation. Therefore, our ability to produce realistic eco-evolutionary predictions relies on quantifications of genetic variances, plasticity and selection at relevant timescales of phenotypic expression and environmental variation. However, such quantifications have rarely been achieved for key traits expressed on short within-year timeframes, especially through early-life stages when eco-evolutionary processes can have strong and long-lasting implications.

Accordingly, we implemented advanced quantitative genetic and selection analyses to quantify early-life potential for evolutionary and plastic responses to environmental changes in the ecologically critical trait of seasonal migration versus residence, using a partially-migratory population of European shags (*Gulosus aristotelis*). First, we devised a quantitative genetic capture-recapture animal model to estimate additive genetic variances underlying migration in autumn and winter for >8,500 juveniles from 11 cohorts. We found substantial additive genetic variances and

resulting heritabilities in autumn, but much smaller values in winter. Meanwhile, parallel analyses showed that selection on juvenile migration versus residence also varies on fine temporal scales, with weak selection in autumn, but strong and fluctuating selection during winter. Together, these results reveal that expression of genetic variances and strong episodes of selection on early-life migration are seasonally decoupled, thereby strongly limiting predicted micro-evolutionary change.

We then developed a general framework to rationalise the dynamics of selection on plasticity expressed in fluctuating environments. This framework considers traits expressed as reversible dichotomous phenotypes, and integrates the critical distinction between selection on plasticity and selection on resulting phenotypes, while incorporating full selection dynamics across temporal sequences of phenotypic expression and environmental variation. We applied this framework to quantify survival selection on early-life plasticity in migration versus residence, by fitting a novel multi-state capture-recapture memory model to fine-scale resighting data from 13 juvenile shag cohorts. We revealed strong and consistent directional selection against early-life plasticity, manifested as substantially lower survival after phenotypic switches from resident to migrant. Yet, this strong selection against plasticity was greatly attenuated across phenotypic sequences expressed from fledging through winter, thereby revealing how episodic costs of plasticity can be absorbed into fitness variation arising in varying environments.

Together, this integrated set of analyses unveils major constraints on rapid evolutionary change in nature, and shows that our ability to predict how populations can adapt to rapidly changing seasonal environments can be notably improved by dissecting eco-evolutionary processes at within-year temporal scales.



Disentangling the demographic drivers of reproductive rate evolution in a wild bird population

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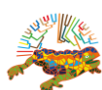
Amid efforts to understand contemporary evolution in the wild, biologists have struggled for decades with the ‘paradox of stasis’: heritable traits showing little or no evolutionary change despite strong natural selection. We here quantify the evolutionary dynamics of clutch size in an island study of nestbox-breeding great tits (*Parus major*) throughout the 20 years following experimental reversal of a spatial genetic distinction between the breeding populations in the East and West parts of the island’s woodland habitat. In particular, we (i) assess the correspondence between predicted and inferred annual adaptive changes to determine the overall reliability of predictions, and (ii) partition evolutionary change among the contributing demographic processes, thereby placing local adaptation of clutch size within its broader evolutionary context.

Comparing predicted and inferred adaptive (i.e., in situ) evolutionary change across all years revealed a dichotomy of bias: not only were responses to selection overestimated in years of relative genetic stasis (the paradox of stasis) but equally enigmatic is that responses were underestimated in years of above average genetic change, such that the paradox of stasis may be the more frequently reported facet of a broader inability to reliably characterise adaptation-in-action. Our diagnostic assessment of this broader predictive failure pinpoints unobserved selection as being the root cause.

However, while inferred responses to selection can inform us of the accuracy of

our forecasts of local adaptation, responses to selection may be overwhelmed by other evolutionary processes and we may thus risk a blinkered characterisation of a trait’s evolutionary trajectory if we do not consider its broader eco-evolutionary context. Indeed, while the post-experimental evolutionary changes were consistent with an adaptationist expectation that perturbation of a key life-history trait will be opposed by natural selection, our demographic decomposition approach revealed that the evolutionary recoveries of both populations towards their pre-experimental states were actually attributable to immigration. Thus, even in the context of a deliberate evolutionary perturbation that was expected to induce natural selection, the contemporary evolution of a key life history trait is explicable only when considering gene flow.

Capitalising on the deliberate evolutionary perturbation of reproductive rate, our study suggests the paradox of stasis is attributable to a combination of analytical and biological challenges that frequently apply to the study of natural populations, but which are ultimately all attributable to an inflated perception of the role of natural selection in local trait evolution. By showing how the contributions of mortality, local recruitment and immigration to genetic change can be disentangled, our analyses clarify that dispersal dynamics must be accommodated if we are to start generating useful predictions of contemporary evolution in the wild.



Effects of urbanization on selection, local adaptation, and eco-evolutionary feedbacks

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Urbanization is causing unprecedented ecological and evolutionary change worldwide. However, it remains unclear what aspects of urban environments cause evolution, whether these factors result in local adaptation, and whether these evolutionary changes drive eco-evolutionary feedbacks within ecological communities. Here, we conducted a reciprocal transplant experiment using white clover (*Trifolium repens*) from urban and rural populations in 5 urban common gardens and 5 rural common gardens. Half of the plants in gardens produced the antiherbivore chemical defense hydrogen cyanide (HCN), and the other half lacked the defence, since this trait is known to exhibit genetic clines along urbanization gradients. We measured multiple fitness traits and ecological interactions with herbivores, pollinators, and

mutualistic root bacteria. We detected divergent selection on HCN between urban and rural environments, where HCN improved fitness in rural environments and reduced fitness in urban environments. Furthermore, there was genetic divergence between urban and rural white clover populations that drove a tradeoff in life history strategies, whereby urban plants invested more in vegetative growth whereas rural plants produced more flowers and seeds. Finally, we demonstrate eco-evolutionary feedbacks, with increased herbivory at rural sites, and increased pollinator visitation to acyanogenic plants at urban sites. This study contributes to understanding how urbanization affects evolution and feeds back to influence broader ecosystem processes.



Environment-specific additive genetic variances for adult survival reveal dramatic contrasts in adaptive evolution across highly fluctuating conditions

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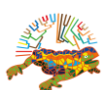
As anthropogenic changes rapidly transform environments worldwide, it is increasingly imperative to predict adaptation underway in wild populations. Key to this objective is quantifying the additive genetic variance for fitness, which represents the net evolutionary response to selection in terms of population growth. In principle, adaptation to stressful conditions caused by unusual environments could be facilitated by the release of cryptic genetic variation that is not expressed under more benign conditions experienced in typical environments. Alternatively, constraints of phenotypic development might restrict genetic variance, and hence adaptive potential, under the most challenging conditions. Additionally, shifts in mean vital rates may alter the extent to which available additive genetic variation actually translates into fitness variation. However, these potential outcomes have rarely been addressed in wild populations experiencing dramatic environmental fluctuations, precluding robust predictions of evolutionary dynamics and rescue.

Accordingly, we quantified the dynamics of additive genetic variance for adult survival in a population of European shags (*Gulosus aristotelis*) that experiences stochastically varying years with benign or extreme conditions (generating a range of annual mortality rates spanning 5–80%). We devised and applied advanced Bayesian analyses combining capture-recapture and animal models to quantify genetic variances for adult survival within and across environmental and biological scales, using 25 years of resighting and pedigree data from ~3500 ringed individuals. Specifically, we enacted the threshold trait model to obtain liability-scale estimates of additive genetic variances. We then transformed these estimates onto the original

dichotomous phenotypic scale of survival versus death, and the continuous scale of longevity.

We detected modest additive genetic variance in liability for survival estimated across all years (heritability, h^2 : 4% of total variance). However, additive genetic variance was three times larger when estimated only across benign years with high overall survival (h^2 : 12%). Yet, this translated into low additive genetic variance on the dichotomous survival scale (h^2 : 2%), but a large emerging genotype-by-environment interaction ('G×E') variance (41%) representing plastic responses to current conditions experienced by each individual. This further translated into substantial additive genetic and G×E variances for longevity (h^2 : 26% and G×E: 28%). Meanwhile, no additive genetic variance for survival was detected across years with extreme conditions. Nonetheless, the discrepancy between estimates depending on whether or not all years were grouped together suggested (if anything) a negative genetic correlation between benign and extreme conditions.

These results support the proposition that adaptive potential is highly restricted in the most challenging environments. They imply that there will be weak evolutionary responses to increasingly frequent extreme conditions, and the negative genetic correlation indicates that any such responses will oppose adaptation to benign conditions occurring otherwise. Yet, the notable additive genetic variance in benign conditions clearly potentiates effective and rapid population recovery after occasional extreme conditions. Overall, by revealing patterns that are hidden to analyses neglecting environmental fluctuations, our study overturns standard expectations about contemporary adaptation in a key fitness component, thereby opening new paths to better predict evolutionary resilience in a changing world.



Evolutionary rescue and critical genetic variance in stage structured populations.

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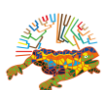
Background: Climate change is driving widespread maladaptation and population decline, pushing many species toward extinction. Threatened populations can avoid extinction through rapid adaptation, a mechanism known as evolutionary rescue. Previous theory has showed that initial genetic variance in fitness increases the probability of evolutionary rescue as it speeds up evolution rate and that there is a critical amount of variance below which rescue is unlikely. Many endangered populations are composed by individuals in different stages, which express different life history traits with different contribution to the population growth rate as measured by demographic elasticities. Elasticities contribute to shaping selection strength on life history traits and to how evolution of these traits impacts population demography. However, only a few studies of evolutionary rescue considered this stage heterogeneity and most of them assumed that environmental change affects a single life-cycle transition, often juvenile survival. Here we predicted evolutionary rescue probability of a red-listed endangered species with a complex life cycle, *Centaurea corymbosa*, following the evolution of the different life history traits of the life-cycle.

Materials and methods: We used 25 years of monitoring of 6 populations of *C. corymbosa* to parameterize a model of evolution in a stage-structured population using the quantitative genetics theoretical framework developed by Barfield et al. (2011). We assumed a Gaussian relation between each life-history trait and an associated latent trait. We simulated the evolution of the population growth rate following genetic variance introduction assuming no initial genetic variance and different amounts of environmental variance in the natural populations. We investigated how the distribution of the introduced genetic variance for different life history traits influences rescue

and long-term population persistence in two scenarios. In the first one genetic variance was introduced for a single life history trait, while in the second (more realistic) variance was introduced for all traits of the life cycle.

Results: We derived analytical predictions that link the genetic variance for the different life-history traits and their elasticities to the rate of change of the population growth rate. When genetic variance is introduced for a single life history trait there is a range of variance that allows rescue. Consistent with our analytical predictions the minimum amount of genetic variance allowing rescue decreases as the elasticity of the life history trait increases. This result generalizes to the multivariate case where evolutionary rescue is more likely when the structure of the genetic variance-covariance matrix translates into increased variance for life-history traits with high elasticity. Further, stabilizing selection induces a variance load which reduces population growth rate and increases with the amount of introduced variance. There is therefore a threshold amount of introduced variance above which population declines to extinction. We finally identified cases where the extinction of some populations of *C. corymbosa* is very likely despite the introduction of variance.

Conclusions: Using a method that only requires life-history trait measurements and assumptions regarding initial genetic and environmental variances, we modeled adaptive and demographic responses of stage-structured populations following genetic variance introduction. Long term persistence is more likely when genetic variance is introduced for life history traits with large contribution to population growth rate. By integrating demographic and genetic factors, our framework aims to improve evolutionary forecasts for conservation decision-making.



Founders predict trait evolution and population dynamics after evolutionary rescue

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Evolutionary rescue helps populations survive environmental change, but the phenotypic and demographic factors associated with rescue dynamics and its long-term effects remain unclear. We experimentally evolved 10 source populations of flour beetles from across India in a suboptimal corn resource for 70 generations (~5 years), collecting >10,000 population census points book-ended by measurements of fitness-related traits for 30 experimental lines. Despite clear ancestral trait differences, all lines showed highly parallel evolutionary rescue within ~20 generations. Long-term population size

varied across source populations and was positively correlated with ancestral development rate, which increased convergently across populations and emerged as the single best predictor of population performance during and after evolutionary rescue. Notably, population dynamics during rescue were uncorrelated both with ancestral trait distributions and post-rescue adaptation. Our results support prior work showing founders as key predictors of adaptation, highlight the role of founder traits for long-term adaptation and trait evolution following evolutionary rescue.



Inbreeding depression throughout the growth period of wild Swiss barn owls

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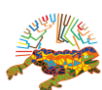
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Selection against inbred individuals – i.e. inbreeding depression – can have severe consequences for the survival of a population. Predicting the effects of inbreeding within populations requires an accurate estimation of the strength of inbreeding depression, which is particularly challenging in wild populations as both fitness and genomic/pedigree data are needed. Here, we use whole genome sequence data from >3,000 wild barn owls in a long-term study population in Switzerland to investigate the presence and change in the strength of inbreeding depression over the course of an individual's life. Using repeated measurements of tarsus length, bill length and mass we modelled the early growth of individuals as a Gompertz curve and ran non-linear hierarchical mixed models to assess the impact of inbreeding on two parameters of the growth curve: growth rate and the final

trait value (asymptote). We show that inbreeding depression can affect both parameters and is expressed not only in the final trait value, but also during the period of growth in some traits. Moreover, we find that such inbreeding depression may be undetected if standard linear mixed model approaches are used because inbreeding depression is present only at certain life stages. Finally, we also show the heritability of traits changes over the course of life, with environmental factors generally contributing more to phenotypic variation in early life which could explain changes in the strength of inbreeding depression we observe in certain traits. Overall, we provide a comprehensive overview of the effects of inbreeding in this wild population, highlighting the dynamic interplay between environmental influences and the selection pressure against inbred individuals.



Not just farther, but more variable: Intraspecific variation of seed dispersal strategies in *Arabidopsis thaliana* as a potential adaptive trait

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Seed dispersal is a crucial ecological and evolutionary process that influences gene flow, population dynamics, and species persistence. While dispersal is mostly studied across species, there is growing recognition of the importance of intraspecific and individual-level variation in dispersal. A key example comes from dispersal kernels, where estimates based on individual-level variation can differ significantly from those based on population means. However, the sources, magnitude, and ecological-evolutionary consequences of intraspecific variation in dispersal remain poorly understood, limiting its integration into both ecological and evolutionary models. In this study, we address four key questions: (1) Do dispersal kernel parameters vary within species? (2) Are these parameters heritable and genetically based? (3) Which plant traits drive variation in each kernel parameter? (4) Can such variation reflect adaptive strategies under different environments?

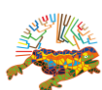
We used *Arabidopsis thaliana* as a model system and conducted wind tunnel experiments to measure seed dispersal distance of 466 individuals from 108 natural accessions. For each individual, we fit a Generalized Normal distribution to the observed dispersal distances and extracted four kernel parameters: mean, standard deviation, skewness, and kurtosis, each capturing distinct aspects of the dispersal strategy. We also measured key morphological and reproductive traits related to dispersal, including plant height, seed yield, silique morphology, seed shape and its within-individual variance. We used Random Forests to identify trait predictors of kernel parameters. We estimated heritability (h^2),

explored environmental associations, and performed GWAS.

We found substantial intraspecific variation and heritability in all kernel parameters ($h^2 = 0.2\text{--}0.5$), supporting a genetic basis. Mean distance, skewness, and kurtosis were primarily driven by plant height and seed yield, traits known to influence long-distance dispersal. In contrast, standard deviation, representing within-individual dispersal variance, was associated with different predictors: seed size, its variance, and silique resistance. We also found that standard deviation was the only parameter that had a significant relationship with the climatic unpredictability index. While GWAS did not identify strong-effect loci on the kernel parameters, polygenic modeling with Bayesian Sparse Linear Mixed Models (BSLMM) highlighted polygenic contributions.

Our study reveals that dispersal kernel parameters vary widely within *Arabidopsis thaliana*, are partially heritable, and are shaped by distinct plant traits. We propose that dispersal kernels, as characterized by their full suite of parameters, represent biologically and ecologically meaningful traits, potentially shaped by natural selection. Our findings contribute to a broader understanding of seed dispersal strategies as complex, potentially adaptive traits, with individual variation in dispersal variance playing a notable role. This study underscores the importance of moving beyond mean dispersal metrics toward a more nuanced, trait-based perspective that considers dispersal as a multivariate and evolvable strategy.

Keywords: Intraspecific variation, seed dispersal, dispersal kernel, dispersal traits, *Arabidopsis thaliana*.



Repeatability of evolution is similarly and additively affected by variation in selection and variation in drift in a heterogeneously replicated experimental evolution study.

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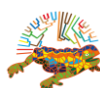
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MEETING ROOM 114, August 21, 2025, 2:00 PM - 5:00 PM

The repeatability of evolution is fundamentally important for understanding the origin and diversification of life as well as for developing evolutionary forecasting tools. A wide-spread assumption is that evolution is more repeatable and more predictable when selection dominates drift in changing genotype, phenotype, and fitness. However, neither selection nor drift are constant in space and time, as both environments and demography vary. Environmental stochasticity results in changing selection coefficients while demographic stochasticity results in changes in the potential for drift. To start exploring the sensitivity of evolutionary predictions to these forms of stochasticity we should assess how outcomes of repeated evolutionary events diverge in response to variation in selection and drift. We replicated an evolution experiment exposing genetically variable *Caenorhabditis elegans* to a novel microbial prey for 20 generations across five institutes in The Netherlands and Belgium. Replication across the institutes led to variation in selective environments: we show that worms exposed to the same primary axis of selection in different institutes have divergent microbiomes and that microbiome variation has an effect on fitness evolution. We also applied different life history and

demographic treatments, including variation in population size (bottleneck), in reproductive modes, and in potential for gene flow. These demographic treatments introduced variation in drift. We found high among-institute variation in fitness evolution, unrelated to variation in genetic diversity but explained by variation in microbiota. Based on nearly neutral theory we derive the theoretical expectation that for adaptation from standing genetic variation, variation in selective environments (among-institute variance) and drift (within-institute variance) have additive effects of similar magnitude on repeatability of fitness evolution. We quantify the effect of environmental and demographic stochasticity in our experiments using mixed effect models and confirm this theoretical expectation. Thus, both environmental heterogeneity affecting selection and demographic heterogeneity affecting drift are important in the repeatability of evolution based on standing genetic variation. Our results underline that evolutionary predictions will improve with more accurate measurements of spatio-temporal variation in selection. Our heterogeneous evolutionary experiment also reveals inherent limits to evolutionary predictions in relation to the demographic and life history context.



Simulated heatwaves induce rapid and suprising multidimensional evolution in host-parasitoid communities

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MEETING ROOM 114, August 21, 2025, 2:00 PM - 5:00 PM

Extreme events, such as heatwaves, are increasing in frequency and intensity, exerting immediate and long-term impacts on biodiversity. While such events can act as selective pressures that enhance tolerance to future extremes, the direction and persistence of these evolutionary changes are likely to be modulated by ecological and evolutionary pressures from co-occurring species. Those species will also experience significant compositional shifts in response to extreme events, creating complex community dynamics. Data on simultaneous ecological and evolutionary responses within closely interacting communities are scarce but essential for understanding the constraints on adaptation to increasingly extreme climatic conditions. We subjected naturally assembled *Drosophila*-parasitoid communities from Australian rainforests to a range of simulated heatwave durations. Alongside species turnover, we observed rapid evolutionary changes in multiple

Drosophila species, including increased vulnerability of reproductive performance to thermal stress, but enhancement in parasitism resistance and competitive ability. Notably, evolutionary shifts in traits predominantly occurred in communities exposed to relatively milder (but not strong) heatwave treatments, supporting our hypothesis of non-linear evolutionary responses to stress. Our findings reveal the rapid evolution of diverse traits and highlight the maladaptation of thermal tolerance in communities, suggesting that heatwave events may lead to both ecological simplification (an ecological legacy) and increased vulnerability (an evolutionary legacy). These results call for a conceptual shift: altered community contexts driven by extreme events can generate multifaceted selection pressures that shape evolutionary trajectories beyond the direct filtering selection of the events themselves.



Some evolutionary demographic consequences of pollen limitation in flowering plants

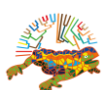
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MEETING ROOM 114, August 21, 2025, 2:00 PM - 5:00 PM

Flowering plants are fascinating for their extreme diversity of reproductive form and function, which vary from combined to separate sexes, outcrossing to self-fertilization, and a staggering array of floral architectures to achieve successful reproduction via pollination. This floral diversity highlights many possible adaptive solutions to a common ecological and evolutionary problem: pollen transport, whether by biotic or abiotic vectors, is an inherently stochastic process that often results in pollen limitation of seed production. Indeed, pollen limitation (e.g., due to variation in pollinator availability) is a widespread phenomenon facing most plant populations in both wild and agricultural contexts. While much attention has been given to the study of how traits influencing male and female fitness components might evolve under pollen-limited conditions, less attention has been paid to the demographic consequences. Indeed, throughout most of evolutionary biology, the assumption that female fecundity limits population growth rates (known as female demographic dominance) is tacitly assumed. I will present

some recent theoretical work in evolutionary demography which explicitly relaxes this assumption to study the joint demographic and evolutionary consequences of pollen limitation for the maintenance of sexually antagonistic genetic variation. The key insight emerging from these models is that, when both ovule and pollen limitation jointly influence population demographic rates, it is not only demographic outcomes that are altered (i.e., population growth rates, persistence, and extinction) but the evolutionary outcomes as well. Specifically we see important changes in the selection conditions favoring the maintenance of genetic variation, male biases in selection, and counter-intuitive effects of altered effective selfing rates. Combining our theoretical models with empirical demographic data suggests that these effects are likely to be important in natural populations, and underscores the importance of carefully considering the ecological details of reproduction in order to understand the evolution of floral traits.



The disperser's gambit: playing the long game in common lizard evolution

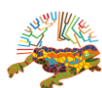
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MEETING ROOM 114, August 21, 2025, 2:00 PM - 5:00 PM

Dispersal polymorphism is widely observed across taxa and populations, despite its close association with individual fitness. To explain the maintenance of polymorphism across generations, numerous hypotheses have been proposed, including frequency-dependence, fluctuating selection, trade-offs with life-history traits, inbreeding avoidance and assortative mating. However, several studies have reported that dispersers in natural populations experience reduced fitness, affecting both reproduction and survival. This raises a fundamental question: how can dispersal polymorphism persist across generations? To address this question, we investigated the long-term consequences of natal dispersal on fitness over multiple generations. Our study benefits from two unique features: (i) a study model exhibiting short intra-population dispersal distances, the common lizard (*Zootoca vivipara*) and (ii) a pedigree spanning 24 years. In the study population, two behavioral phenotypes associated with natal dispersal were identified: residents and dispersers. We inferred long-term fitness associated to both phenotypes by conducting a gene dropping analysis using the pedigree, simulating gene segregation among relatives to track both the genetic contribution of each individual and their lineage longevity. We defined the expected genetic contribution as the expected number of descendants carrying part of an individual's genetic make-up, while lineage longevity represented the duration over which an individual's genetic make-up persists within the population. Our results demonstrate that natal dispersal status shows moderate heritability ($h^2 = 0.192$ [2.033e⁻¹⁰; 0.419]) in our study

population, suggesting potential for evolutionary change. Our findings further reveal that natal dispersal induce short-term fitness costs, including lower probability of accessing reproduction and reduced reproductive success. However, we found that, in the long-term, the genetic contribution of residents and dispersers are similar; both strategies appear effectively neutral relative to each other. We show that this equilibrium can be explained by the fact that dispersers produce longer-lasting genetic lineages than residents, suggesting long-term, inter-generational benefits that counterbalance immediate individual costs. The most supported hypothesis explaining these results is that dispersers exhibit a lower lifetime reproductive success compared to residents, but their descendants achieve greater fitness than those of residents, ultimately producing more offspring. We will discuss potential mechanisms underlying this phenomenon, particularly focusing on why the descendants of dispersers may preferentially access reproduction. Without long-term fitness metrics enabled by gene dropping analysis, such balanced selection between the two strategies would have remained undetected, leading us to conclude to a case of “paradox of evolutionary stasis”: an absence of phenotypical change over generation despite strong selective pressures. We thus believe such long-term analysis of fitness can deepen our understanding of adaptation, and we encourage further studies to utilize such techniques for other traits. We hypothesize that this approach may provide new insights for the “paradox of evolutionary stasis”.



The quest for life-history trade-offs continues: Building on the Y-model of resource acquisition and allocation to reveal the lifespan cost of reproduction

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MEETING ROOM 114, August 21, 2025, 2:00 PM - 5:00 PM

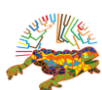
Although central to life-history theory and evolutionary models of ageing, the predicted trade-off between reproduction and survival remains remarkably elusive on both the phenotypic and the genetic level. Indeed, a recent meta-analysis concluded that genetic correlations among life-history traits are – on average – positive rather than negative, which led the authors to suggest that trade-offs are less common than typically assumed (Chang et al 2023). Another meta-analysis, focussing specifically on the association between reproduction and survival, recently came to a similar conclusion (Winder et al 2025).

Here we build upon classic work (e.g. the Y-model of Van Noordwijk and De Jong, 1986), that emphasises the roles of variation in resource acquisition vs. allocation in shaping life-history correlations. In particular, we will distinguish between genetic and environmental sources of variation in resource allocation and acquisition, and how these relate to the widely-used, yet contested concepts of condition, quality, and fitness. Furthermore, we emphasise the difference between phenotypic and genetic patterns of covariance on the one hand, and the trade-off function that describes the relationship between resource allocation to, for example, reproduction versus survival, on the other. We conclude that while phenotypic and quantitative genetic correlative data may provide a comprehensive description of the role of trade-offs in shaping the status quo, they tell us little about their role in shaping

the evolutionary trajectory that led the population there.

We illustrate this using empirical studies of three contrasting systems—humans, quails and great tits—that aimed to infer and quantify the trade-off function between reproduction and lifespan. Using complementary approaches, we show how women reproducing when resources are scarce, great tits genetically predisposed to lay large clutches, and quails artificially bred to invest more in reproduction all live shorter lives. Thereby we provide strong empirical evidence for a lifespan cost of reproduction across a range of life-history strategies.

In conclusion, we demonstrate significant lifespan costs of reproduction, and how these can easily go undetected. Furthermore, we highlight the limitations of correlative attempts to quantify the importance of trade-offs, and how phenotypic and genetic correlations—or their absence—provide an incomplete picture of the importance of trade-offs in shaping past and future evolutionary adaptation. Hence, we call for a more nuanced perspective on the role of trade-offs and their importance in shaping patterns of standing (co)variation, and how these are reflective of evolutionary constraints. We argue that this requires a plurality of approaches, and clear and unambiguous terminology that captures variation in resource acquisition and allocation, their underlying genetic and environmental sources, and how these manifest themselves in wild populations.



Using the quantitative genetic roadmap to predict responses to selection in wild populations; from theory to empirical examples of environmental shortcuts in wild roe deers, clownfish, and snapdragon plants

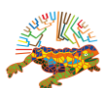
Pujol B¹, Gervais L¹, Salles O¹, Marrot P¹, Thomson C¹, Mougnot P¹, Gourcilleau D¹, Mousset M¹

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MEETING ROOM 114, August 21, 2025, 2:00 PM - 5:00 PM

Although there are many examples of contemporary directional selection, evidence for responses to selection that match predictions are often missing in quantitative genetic studies of wild populations. This is despite the presence of genetic variation and selection pressures that are the theoretical prerequisites for the response to selection. This conundrum can be explained by statistical issues with accurate parameter estimation, and by biological mechanisms that interfere with the response to selection. These biological mechanisms can accelerate or constrain this response. These mechanisms are generally studied independently but might

act simultaneously. I will present these mechanisms and discuss their potential combined effect. I will also present quantitative genetic results obtained in wild populations of Roe Deer, Clownfish, and Snapdragon plants, showing how the environment may shortcut selection through the environmental similarity and spatial autocorrelation of traits and fitness. These results, some of which are preliminary, have implications for evaluating whether wild populations have the evolutionary potential to respond to selection or whether they are at the mercy of the environment. They also have implications for explaining the apparent evolutionary stasis of wild populations.



When selection doesn't add up: non-additive evolutionary responses to multiple selective pressures in *Drosophila melanogaster*

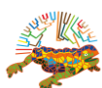
Montbel V¹, Ravi Kumar V¹, Rasouli-dogaheh S¹, Fedorchenko V¹, Hrček J¹

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MEETING ROOM 114, August 21, 2025, 2:00 PM - 5:00 PM

Natural selection results from a great number of biotic and abiotic selective pressures acting simultaneously on populations. These pressures often interact, leading to non-additive outcomes where adaptation is not a simple sum of individual selection effects. This study aims to understand the complex evolutionary dynamics of populations subjected to multiple selective pressures, as is typical in natural communities. Using an experimental evolution approach, we exposed populations of *Drosophila melanogaster* to three ecologically relevant selective pressures: high intra-specific competition, heat waves, and parasitoid attacks by *Leptopilina boulardi*. These pressures were applied individually and in combinations over 15 generations. To quantify evolutionary responses, we measured fitness traits every five generations, specifically assessing viability, fertility, parasitoid resistance, competitive ability, and heat-shock resistance. Additionally, genomic changes were assessed using whole-genome pool sequencing. We observed evolutionary responses at both genomic and phenotypic levels under all selective pressures. However, combinations of the selective pressures produced complex, non-additive outcomes. For instance, adaptation to heat stress was constrained when combined with high competition or parasitism. Conversely, parasitoid resistance increased under heat-wave conditions, potentially due to a shared

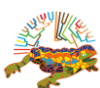
stress response pathway. Such outcomes suggest the presence of trade-offs or underlying genomic covariance that constrain adaptation across multiple traits in some cases and facilitate it in others. Interestingly, the nature of non-additive effects depended more on the traits measured than on the specific combinations of selective pressures. For example, we observed antagonistic interactions for parasitoid resistance and heat-shock resistance, whereas competitive ability displayed more additive responses. Additionally, the strength and direction of evolutionary responses varied across replicates, depending on the number and identity of selective pressures, highlighting the role of stochasticity in complex environments. These findings demonstrate that interactions among selective pressures can either buffer or amplify evolutionary change in ways that are trait-specific and difficult to predict. Such complexities reveal the limitations of traditional single-pressure studies in predicting evolutionary outcomes in natural settings. Understanding these non-additive dynamics is essential for accurately predicting adaptive potential in the face of environmental changes, such as climate shifts or invasive species. Our study emphasizes the need for more integrative experimental evolution designs that better reflect the multidimensional nature of natural selection.





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S43 - The dynamics and consequences of bacteria-bacteriophage interactions and co-evolution in complex communities



Eco-evolutionary dynamics of phage-bacteria interactions in the human gut through the analysis of CRISPR arrays

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POSTER SESSION 1 - P01.337, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Interactions between viruses and microbes are among the most relevant and least understood forces driving microbiome dynamics. Such interactions can be monitored through the study of CRISPR arrays, the component of the CRISPR-Cas prokaryotic immunity system that stores short fragments of DNA from viruses and other mobile genetic elements when they are encountered. Using that approach, we characterized the dynamics of phage-bacteria interactions and the effects of CRISPR-Cas immunity in a large-scale longitudinal study of the human gut microbiome.

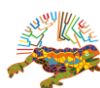
We first developed an efficient computational workflow to assemble around 80,000 CRISPR-Cas loci from metagenomic samples, identified and annotated the targets of CRISPR immunity, and quantified the fluctuations in their abundance. Then, we studied how phages affect the microdiversity of bacterial populations and how the abundance of different phages and plasmids

responds to the acquisition of matching sequences in their hosts' CRISPR arrays.

Our analyses reveal that phages impose a selective pressure that favors host lineages with protective CRISPR arrays; however, acquisition of immunity does not produce complete selective sweeps in the host population. Conversely, CRISPR immunity at the whole-microbiome level significantly decreases the abundance of lytic phages in the gut, but rarely leads to their complete elimination. A much smaller effect is observed for lysogenic phages and plasmids.

Finally, we present evidence of selection for long-lasting immunity against endemic phages, as demonstrated by an enrichment in spacers targeting local lysogenic phages in distal (and chronologically older) regions of CRISPR arrays [1].

[1] López Beltrán, Botelho, Iranzo (2024) ISME J. 18(1):wrae134



Spacer Chronicles: Reconstructing ancestral CRISPR Spacer Arrays to Reveal Phage-Host Dynamics

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POSTER SESSION 1 - P01.336, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

In the perpetual arms race between phages and their prokaryotic hosts, CRISPR systems serve as adaptive defense systems within prokaryotic genomes. They are composed of CRISPR supporting genes (Cas) and CRISPR arrays, highly diverse and categorized into a multitude of types.

To protect the cell, short snippets of DNA, called spacers, are transcribed from invading DNA by Cas proteins and incorporated in the CRISPR array within the cell's genome. Subsequently, Cas proteins incorporating the spacers can interfere with the invaders thereby providing immunity.

Remarkably, new spacers are (almost) always inserted at one end of the array, the leader end, and thus the sequencing of spacers within an array provides a chronology of invasions by phages and plasmids. Due to their repeat structure, spacers may also be deleted as a result of misalignment during recombination or mutations. Thus CRISPR arrays are shaped and rapidly evolve through acquisition and deletion of spacers.

Therefore, studying the evolution of CRISPR array and reconstructing their ancestral spacer composition enables us to study the interaction of prokaryotes with phages, plasmids and mobile genetic elements through time and consequently gives insight into both the composition of the metagenome and conflicts between its components.

However, ancestral reconstruction tools that are commonly used are inadequate for reconstructing ancestral CRISPR arrays. In particular, these tools fail to consider the order of spacer acquisition. Thus, we

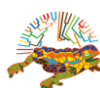
introduce a new tool, SpacerPlacer, that identifies evolutionary patterns of a group of closely related CRISPR arrays and respects the spacer acquisition order. We estimate acquisition and deletion events along their phylogenetic tree and thus the CRISPR arrays ancestral states.

The reconstruction of the ancestral states of CRISPR arrays allows us to explore their evolutionary behavior. We find that spacer deletions are on average 374 more frequent than mutations and are often deleted in multiples, and spacers are less often deleted at both ends of the array.

Additionally, SpacerPlacer enables the prediction of the leader end of the array and thus draw conclusions about the transcription orientation.

We analyzed a large database of CRISPR arrays of a wide diversity of CRISPR-Cas types to discover their individual (evolutionary) differences and quirks. In particular, some types can spread their defensive spacer repertoire across multiple CRISPR arrays, some types clearly prefer to co-occur with other types likely lending supporting each other in acquisition and interference processes or carry out independent and complementary functions in the cell.

Moreover, besides improving our understanding of CRISPR array evolution, SpacerPlacer allows us to leverage the highly specific one-to-one correspondence between phages/plasmids and spacers to identify and track individual spacers of interest, discover selection patterns and thus explore the ecology and co-evolution of groups of bacteria and their phages.



Surprising morphological and genetic diversification during experimental evolution of phage M13

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POSTER SESSION 1 - P01.339, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Although viruses are often thought of as solitary, many are in fact highly cooperative and susceptible to cheating. Cheats have been characterized especially well in the non-lytic, filamentous phage M13, with many independent evolution experiments showing the same pattern: cheats emerge readily via mutations that delete genes for producing public goods, become abundant, and then exhibit dynamic cyclical oscillations as they coevolve with cooperators. We experimentally evolved a strain of M13 under conditions known to select for cheating. Instead of observing cheats similar to those previously described in the literature, we observed remarkable diversification in the absence of any clear adaptive advantage. Evolved populations were dominated by

mutants that formed plaques with varying degrees of turbidity (morphological diversification); most evolved mutants carried unlinked, unique single-nucleotide mutations (genetic diversification); the turbid plaque morphologies rapidly proliferated before coexisting stably with wild-type plaques (maintenance of diversity); and none of the evolved mutants could significantly outcompete the wild-type ancestor (no fitness advantage). These puzzling results highlight opportunities for using filamentous phages to tackle broad evolutionary questions, from the evolution of cheating (why do cheats sometimes fail to evolve) to questions of diversification (how can such diversity be stably maintained)?



Temperature extremes alter phage predation dynamics and the capacity for bacterial coexistence

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POSTER SESSION 1 - **P01.338**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Predation, particularly frequency-dependent predation, plays a crucial role in shaping species interactions and promoting coexistence in microbial communities. However, temperature fluctuations, especially extreme temperature events, can alter the dynamics between predators and prey, potentially destabilizing coexistence. Previous theoretical models suggest that, under certain conditions, predators may be more sensitive to temperature changes than their prey, leading to shifts in community structure where competition may outpace predation. In this study, we investigate the effects of heat treatments, both steady and stochastic, on the coexistence of two *Pseudomonas* species in the presence of their phage predators. Our goal is to understand how temperature-induced changes in phage dynamics influence bacterial coexistence.

Materials and Methods: We established a two-species bacterial community composed of the weak competitor *Pseudomonas putida* MM1 and the strong competitor *Pseudomonas protegens* CHAO, the latter of which produces a suite of antimicrobial secondary metabolites when in co-culture. These bacteria were exposed to specialized phage predators, Psp1 (for *P. putida*) and ϕ GP100 (for *P. protegens*). To explore the effects of temperature, we conducted a 9-day serial transfer experiment under three different temperature regimes: (1) ambient summer temperature, as recorded by MeteoSwiss over the last decade in Bern, Switzerland; (2) ambient temperature warmed by 4°C; and (3) ambient temperature with stochastic fluctuations around the daily average using a sinusoidal model in R to simulate temperature variability. Each combination of bacteria and phages was

tested in a full factorial design with six replicates per population across the three temperature treatments, including blanks and bacterial monocultures.

Results: We found that higher temperatures, especially with stochastic fluctuations, reduced bacterial coexistence. Phage performance, particularly for ϕ GP100, was significantly diminished at elevated temperatures, with a marked decrease in phage reproduction rates under more variable temperature conditions. When only one phage is present it reduces severely its prey and allows the other bacterial strain to gain a competitive edge. When both phages are present at ambient temperatures, the bacterial strains can coexist. The bacterial species demonstrate adaptive responses to higher temperatures, and in environments with stochastic temperature changes and with both phages present, the stronger competitor (*P. protegens*) often outcompeted the weaker (*P. putida*). However, when temperatures were increased steadily, phage-driven coexistence was better maintained, suggesting that steady warming may allow for more stable interactions between phages and their bacterial hosts.

Conclusions: Our findings underscore the complex interplay between temperature dynamics and microbial community composition. The type of temperature change, steady or stochastic, has significant implications for the stability of microbial coexistence, particularly in the context of phage predation as a regulator of competitive species of bacteria. As climate change leads to more frequent and extreme temperature fluctuations, understanding how these changes affect microbial interactions will be crucial for predicting shifts in community structure and ecosystem function.



Understanding phage-host dynamics during the course of a cyanobacterial bloom in the Baltic Sea

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POSTER SESSION 1 - P01.335, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Cyanobacterial blooms are ecologically significant events in aquatic ecosystems, affecting the equilibrium of the microbial community. However, despite the deeply interconnected evolutionary history of phages with their hosts and their significance in modulating their host dynamics, phage-host interactions and dynamics during bloom events remain poorly understood. Here we inspect a cyanobacterial bloom event dominated by *Dolichospermum* in the Baltic Sea with high temporal resolution, applying a multi-omics perspective to unravel the role of phage-bacteria interactions in community dynamics during the bloom event.

To study the phages and cyanobacteria interactions, surface water samples were collected from the Baltic Sea throughout the bloom of *Dolichospermum*, a diazotrophic filamentous cyanobacteria. This sampling captured a daily temporal resolution of the *Dolichospermum* bloom event from initiation to collapse (6 days from 27 Jun 2023 to 2 July 2023). Biomass was collected on filters with in 5 µm pore size (large fraction), and the flow-through water was filtered through 0.1 µm filters (small fraction). These filters were used for DNA extraction and metagenome sequencing. A long-read Nanopore metagenome was also generated. Metagenomes were assembled and binned to reconstruct the microbial community. Virus identification tools, geNomad, Vibrant, and PPRmeta, were applied to assembled contigs to extract viral contigs from metagenomes. Additionally, microbial biomass was collected on filters with 0.8 µm pore size, and the extracted RNA from these filters was used for metatranscriptome sequencing.

A total of 684 metagenome assembled genomes (MAGs) were reconstructed from sequenced metagenomes that were clustered into 181 Operational Taxonomic Units (mOTUs). Representative MAGs from all mOTUs were dominated by representatives of phyla Pseudomonadota (33%), Actinomycetota (28%), Bacteroidota (21%), Verrucomicrobiota (9%), and Cyanobacteriota (5%). While the abundance of cyanobacterial MAGs decreased on day 6, a notable increase in the abundance of almost all other representative MAGs was observed at the collapse of the bloom.

To reveal the diversity of viral populations associated with key microbial taxa during the bloom, we identified viral contigs from metagenomic assemblies. The majority of viral contigs were classified as Caudoviricetes (70%), a class of dsDNA viruses known as the tailed phages. The next most abundant group was Megaviricetes (16%), a class of NucleoCytoplasmic Large DNA Viruses (NCLDV) which are known to infect marine and freshwater eukaryotes like algae and protists.

We will further combine the long-read metagenomics and metatranscriptomics analysis to enable high-resolution genomic characterization of the microbial community, identifying RNA viruses as well as inspecting changes in the metabolic profile of the microbial community throughout the bloom event. This study will enable us to provide detailed insight into the phage-host dynamics during a bloom event and unravel the impact of phage infection on bloom dynamics and metabolic activity of the whole community.



Adaptive strategies during coevolution depends on multipartite interactions between bacteria and their temperate phages.

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MEETING ROOM 120+121, August 19, 2025, 4:00 PM - 5:30 PM

Community dynamics are strongly shaped by the presence of parasites, among which, prophages. These can induce their hosts death, but they can also increase their fitness in numerous ways, including infection of direct competitors. Most works that study bacteria-phage interactions are performed using virulent phages at high titers. This has precluded major advances in the understanding of the multipartite interactions between coevolving bacteria and their resident temperate phages.

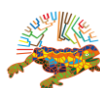
To follow population dynamics, evolution of resistance mechanisms and phage evolution, we performed a coevolution experiment between a phage-sensitive and a polylysogenized strain for over ~200 generations. They were allowed to evolve under different degrees of parasitic pressure: (i) standard conditions, (ii) supplement with citrate to inhibit phage infection and (iii) supplemented with mytomycin C to increase the phage titers. The initially sensitive strain remained in all populations and its frequency was highest when phage pressure was strongest. Contrary to expectation, resistance to temperate phages does not emerge by the incorporation of the phage in the new host (lysogeny). Our experimental results and the individual-based simulations that quantify the cost of lysogeny versus other mechanisms of resistance show that lysogeny is expensive and thus counterselected. At first, most populations become resistant to phages by abolishing the production of the bacterial capsule, a known phage receptor, but eventually capsulated clones increase in frequency owing to the emergence of different resistance mechanisms. Whole genome

sequencing revealed that that many clones accumulated mutations resulting in the modification of capsule production or its biochemical composition to escape predation, without a complete loss of this important structure. Most surprisingly, we observed frequent emergence of resistant phenotypes for which there does not seem to be a genetic basis. These changes are quickly lost in absence of phage pressure and suggest the existence of transient mechanisms of phage resistance that do not require the fixation of costly mutations.

The polylysogenized strain generically evolved to offset the cost of phage outburst by increasing population yield but not growth rate. Interestingly, we observed “division of labor” whereby a subpopulation no longer produced any phage, and could no longer adsorb their own phages. The phage-producing subpopulation could still adsorb phage but the amount of plaques produced depend on life-history. Whereas subpopulations evolved in mitomycin C effectively reduced the number of plaques at the end of the experiment, all other populations produced ca 3 logs more of plaques than the ancestor.

Finally, we also show that phages adapted rapidly, for instance, we observed shifts in infection dynamics through time, as well as the ability to circumvent the blockage of infection imposed by the addition of citrate.

Taken together, we show the intricate relationship between all interacting partners is driven by the cost of each adaptive strategy whence in a community setting. More studies are required to confirm that the principles outlined by this experiment and how they can apply to a broader set of interacting partners.



The phageome of apricot trees and its association with bacterial canker disease

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MEETING ROOM 120+121, August 19, 2025, 4:00 PM - 5:30 PM

While phages have been extensively studied in human and marine contexts, a huge gap exists in plant-associated phage research [1,2]. Most plant-related phage research focuses on biocontrol candidates, neglecting ecological and epidemiological questions. *Pseudomonas syringae* is a highly diverse complex of bacterial species found in a wide range of environments, including plants. Bacterial canker of apricot trees involves several strains of this species complex [3]. The diversity and role of phages in this system remains to be elucidated.

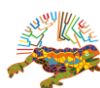
The aim of this study is to define the "phageome" in apricot trees, comparing healthy and diseased tissues. To this end, purification of viral particles and metagenomic analyses were conducted in soil, buds and twigs of apricot trees. Additionally, metabarcoding analyses estimated the diversity of *P. syringae* strains in these samples. In the viral fraction of our purified samples we detected up to 13,111 phage fragments (vOTUs), and 28% were assigned to a bacterial host [4]. The specific richness of phages in the soil was higher than that of the other tree niches, which were equivalent. Phage populations were distinct, with some phages being ubiquitous across all tree niches. Interestingly, we detected the absence of abundant phages of the Actinomycetota and Bacillota phyla in symptomatic twigs, in contrast to the presence of these phages in the other niches. Conversely, there was a significant increase in the relative abundance of *Pseudomonas* phages in diseased twigs relative to healthy ones. Regarding bacteria, buds and asymptomatic twigs had a similar *P. syringae* population structure, while symptomatic twigs have a distinct signature that sets them apart from other substrates. The

soil *P. syringae* genetic richness is higher than in the other niches.

These findings suggest a potential dysbiosis in the phage community associated with bacterial canker disease, where the increase in *Pseudomonas* phages may reflect a population response to bacterial proliferation in infected tissues. The absence of Actinomycetota and Bacillota phages in symptomatic tissues further indicates that disruption of the normal phage-bacterial equilibrium might contribute to disease progression. The present study represents a pioneering investigation of the phageome associated to plant health. This could lead to advances in biocontrol and disease surveillance, potentially offering new ecological approaches to managing bacterial canker by restoring or enhancing natural phage populations that maintain bacterial equilibrium in healthy trees.

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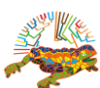




ESEB2025
BARCELONA 17-22 AUGUST 2025

CONGRESS OF THE EUROPEAN SOCIETY FOR
EVOLUTIONARY BIOLOGY

**S44 – The ecological and evolutionary
implications of climate change on
reproduction**



Birds in the Grey Zone: High-Resolution Satellite Imagery Reveals Continental Patterns in Urban Great Tits (*Parus major*)

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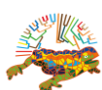
POSTER SESSION 2 - **P02.348**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Urbanization is reshaping ecosystems worldwide, yet its effects on key wildlife traits—such as plumage coloration—have been largely investigated comparing versus rural habitats, while a crucial gap still remains in exploring fine-scale, continuous measures of urbanization to capture subtle variations within the urban mosaic. Carotenoid-based plumage coloration is especially relevant because it reflects an allocation trade-off with vital functions, including antioxidant defence, immune responses, and reproductive success, supposedly more intense in urban habitats due lower dietary availability of, and increased physiological needs for, these pigments.

Here, we demonstrate a continuous negative relationship between urbanization intensity and carotenoid-based plumage coloration in great tits across Europe, leveraging an innovative semi-automated GIS approach with high-resolution (0.1 m) satellite imagery to map impervious surfaces within individual bird territories. Initial findings from a long-term Parisian population showed that higher urbanization

correlated with paler plumage. To test the broader significance of this pattern, we incorporated feather samples from five other European populations (Montpellier, Warsaw, Turku, Malmö, Antwerp). These complementary analyses consistently replicate the Parisian trend, revealing a widespread ecological response to urban environments through the European continent.

Because we distinguish vegetation from impervious surfaces at sub-metric resolution, our methodology addresses critical limitations of coarser land-cover datasets, offering a standardized fine-scale measure of urbanization. This study provides the first continent-wide evidence linking continuous urban parameters directly to continuous phenotypical traits at such a high spatial resolution. Our results further suggest that carotenoid-based plumage coloration could serve as a reliable bio-indicator of urban intensity, with potential applications for monitoring urban environmental health and guiding future conservation strategies.



Breeding phenology drives variation in reproductive output, reproductive costs and offspring fitness in a viviparous ectotherm

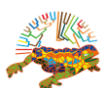
BODINEAU T^{1,2}, de Villemereuil P³, Agostini S⁴, Decenci re B⁴, Le Galliard J^{2,4}, Meylan S²

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POSTER SESSION 2 - P02.349, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Phenological advances are a widespread response to global warming and can contribute to determine the climate vulnerability of organisms, particularly in ectothermic species which are highly dependent on ambient temperatures to complete their life cycle. Yet, the relative contribution of breeding dates and temperature conditions during gestation on fitness of females and their offspring is poorly documented in reptiles. Here, we exposed females of the common lizard *Zootoca vivipara* to contrasting thermal scenarios (cold versus hot treatment) during gestation and quantified effects of parturition dates and thermal treatment on life-history traits of females and their offspring for one year. Overall, our results suggest that parturition date has a greater impact than thermal conditions during

gestation on life history strategies. In particular, we found positive effects of an earlier parturition date on juvenile survival, growth and recruitment suggesting that environmental dependent selection and/or differences in parental quality between early and late breeders underlie seasonal changes in offspring fitness. Yet, an earlier parturition date compromised the energetic condition of gravid females, which suggests the existence of a mother-offspring conflict regarding the optimisation of parturition dates. While numerous studies focused on the direct effects of alterations in incubation temperatures on reptile life-history traits, our results highlight the importance of considering the role of breeding phenology in assessing the short- and long-term effects of thermal developmental plasticity.



Comparison of reproduction-related parameters between free-ranging sex-reversed and sex-concordant agile frog males

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POSTER SESSION 2 - P02.364, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

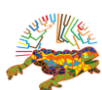
Environmental sex reversal is a phenomenon when environmental conditions experienced during early ontogeny, such as certain temperatures, chemical pollutants or other stressors, override the sexual phenotype otherwise determined by sex chromosomes. A growing body of evidence from free-ranging populations highlight that sex reversal may be common across ectothermic vertebrates, and its frequency is expected to increase under climate warming. Sex reversal can distort population sex ratios and lead to various evolutionary scenarios, depending on the relative fitness of sex-reversed and sex-concordant individuals. Still, empirical data on potential differences between sex-reversed and concordant individuals in free-ranging populations is extremely scarce. The agile frog is an ideal system for studying this problem, because elevated larval incubation temperatures cause sex reversal in genotypic females (XX), and sex-reversal frequency can be high in free-ranging populations. Consequently, males with XX and XY genotypes have to compete for breeding opportunities, but it is unknown if they differ in phenotypic features relevant for intrasexual competition.

We captured hundreds of mature free-ranging male agile frogs at arrival to a breeding pond where sex-reversal frequency is high, and collected buccal swabs for DNA extraction and subsequent determination of the sexual genotype. The frogs were measured for snout-vent length (SVL), forearm width and length, and nuptial-pad length with callipers, because these parameters can influence success in male-male competition for females. We also captured photographs under standard lighting for the measurement of nuptial-pad colour that may be

related to testosterone levels based on previous reports from other species. Testosterone not only mediates gonadal growth, but plays important role in reproductive behaviour as well, and acute stress increases corticosterone levels while may decrease testosterone levels in amphibians. We collected waterborne urine samples from 20 sex-reversed and 20 sex-concordant males for laboratory measurements of testosterone concentration to assess potential differences between the two male categories, and we also measured corticosterone concentrations in the same samples to assess if differences in testosterone levels might be caused by different stress levels (e.g. in response to capturing and handling).

We found no difference in SVL, forearm width and length, nuptial-pad length, nuptial pad colour and urine-corticosterone levels between sex-reversed and sex-concordant males. However, sex-reversed (XX) males had significantly lower urine-testosterone concentration compared to sex-concordant (XY) males.

These latter results suggest potential fitness differences, because testosterone levels can influence several aspects of reproductive behaviour, including engaging in amplexus. To our knowledge, this is the first data on phenotypic differences between sex-reversed and sex-concordant males in free-ranging frogs, and our findings emphasise the need for such studies in the broad taxonomic range where sex reversal occurs. Warming temperatures are expected to increase sex-reversal frequency in various ectotherms on a global scale, which might lead to overall decrease in reproductive capacity if sex-reversed males in general have lower testosterone levels.



Couch potato chipmunks: negative effects of supplemental feeding on breeding activity in eastern chipmunks

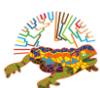
Phendler E¹, Garant D², Tissier M³, Réale D⁴, **Bergeron P**¹

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POSTER SESSION 2 - **P02.356**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Reliable environmental cues contribute to the expression of optimal phenotypes. However, cues governing activity and reproductive phenology may become unreliable due to global changes and invasive pathogens, resulting in mistimed breeding attempts. We have conducted a supplementary feeding experiment buffering a natural pulsed resource on one of three study sites in 2022-2024 to investigate the effects of supplemental feeding on body mass, above-ground activity, oestrus and reproductive phenology in the Eastern chipmunk (*Tamias striatus*). We also aimed to assess the potential reproductive costs associated with plastic responses in activity in this species. Eastern chipmunks are small

hibernating rodents whose above-ground activity and reproductive phenology revolve around masting of the American beech trees (*Fagus grandifolia*). Reproduction typically occurs once a year, in the summer before and the spring following a beech mast. Our results indicate a significant suppression of above-ground activity and reproductive rates in younger supplemented cohorts. The ensuing suppression of above-ground activity has ultimately resulted in missed breeding attempts compared to older supplemented and unsupplemented cohorts. Mistimed resources abundance may thus have unexpected negative effects on breeding activity.



Dark waters, strong beginnings: investigating maternal and environmental influences on early-life traits in Eurasian perch (*Perca fluviatilis*)

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POSTER SESSION 2 - **P02.354**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

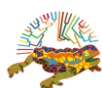
Environmental changes are reshaping freshwater ecosystems, with brownification emerging as a significant threat. This process, driven by increasing concentrations of dissolved organic carbon (DOC), lowers pH, reduces transparency, and alters habitat conditions for aquatic organisms. Increased DOC has complex effects on organisms, which may be especially critical at reproductive and embryonic development stages. Eurasian perch (*Perca fluviatilis*) is a widespread teleost which can reproduce in a wide range of habitats and is one of the few boreal teleosts that has successfully adapted to live in both alkaline and acidic pH, high DOC waters. However, the relative importance of genetic factors that potentially facilitate development in different ionic environments is not well-understood.

In this study, we set out to investigate how DOC and pH influence early-life traits in Eurasian perch embryos, focusing on behavioral (movement frequency), developmental (hatching success) and morphological traits (size) depending on parental populations. The breeders originated from one humic (Kuulma lake) and one clear-water lake (Saadjärv lake) in Estonia. We conducted a partially factorial experiment using pure humic (low pH, high DOC), clear-water (high pH, lower DOC) and inter-habitat crosses where full- and half-sib families developed in water treatments which differed in pH (~4.5, ~5.5 and ~7.5) and DOC (0 mg/L, 50mg/L).

Results showed that maternal population is particularly important for shaping early-life

traits such as hatching success and others across treatments. In both the natural and artificial water experiments, embryos originating from the humic-water population consistently exhibited higher movement levels than those from the clear-water population, indicating distinct behavioral differences between the two groups. Interestingly, water treatment alone (i.e., changes in DOC and pH) did not significantly influence embryo movement nor egg mortality. However, a significant interaction between parental origin and water treatment was observed for hatching success, emphasizing the role of genetic and maternal background in mediating environmental responses. Higher movement levels were also associated with increased egg mortality, suggesting a potential trade-off between activity and survival. These results indicate that behavioral and developmental responses are not solely driven by environmental conditions such as DOC or pH but are also shaped by population-specific traits.

Overall, our findings reveal the complex effects of increased DOC and acidic pH on early-life traits in Eurasian perch. The population-specific responses, particularly in hatching success and movement activity, emphasize the importance of maternal and genetic background. These insights improve our understanding of how fish populations may respond to ongoing environmental change, potentially informing conservation and management strategies in increasingly humic and acidic freshwater ecosystems.



Does temperature affect the adaptive value of male harm in *Drosophila melanogaster*?

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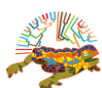
POSTER SESSION 2 - **P02.361**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Male harm is a widespread phenomenon, by which interacting with males reduces female fitness. Male harm has consequences for populations, affecting adaptation, evolutionary rescue, demography and population viability. The persistence of male harm, despite its costs, is largely attributed to the idea that harmful traits are adaptive for males. However, the adaptive value of male harm has rarely been tested. Furthermore, recent evidence suggests that ecology can modulate the effects of male harm via both plastic responses within generations and adaptive responses over evolutionary timescales. Here, we investigate the relationship between male reproductive success and ability to harm females, across a natural thermal range.

We derived 29 isogenic lines from a wild population of *Drosophila melanogaster*, and set up assays to measure male harm and male reproductive success at three different temperatures (20°C, 24°C and 28°C). To

measure male harm, we exposed individual females to either 1 male (low harm) or 3 males (high harm) from each isogenic line, and measured the difference in the lifetime reproductive success of these females. To assay male reproductive success, we allowed individual focal males from each isogenic line to compete with 2 rival males for reproduction with 3 females. We estimated the fitness of the focal males as their paternity share in the competitive mating environment, measured over their lifetime.

We first used these data to examine the reaction norms for male harm and male fitness across a thermal range, to elucidate the extent of phenotypic plasticity in these traits. Next, we examined the sign and strength of the correlation between male harm and fitness, at each temperature in our thermal range, to infer how this relationship varies.



Dynamics of Mutation Load in the Antarctic Fur Seal (*Arctocephalus gazella*)

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POSTER SESSION 2 - P02.355, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

A major factor determining the fitness of an individual or population is constituted by their genetic load.

In particular, theory predicts that the amount of deleterious mutations influences the resilience of populations that are declining due to anthropogenic impacts.

In the wild, the effect of deleterious mutations is coupled to their demographic context, as populations size impacts both the degree of inbreeding and the effectiveness of selection.

Therefore, a population's history influences if genetic load is exposed to selection, as well as how effectively selection can purge deleterious mutations from the gene pool.

Conversely, the degree of mutation load effects the degree of inbreeding depression that a population is faced with when its population size is declining.

The magnitude of mutation load therefore has direct implications for a population's persistence in changing environments.

Understanding the fitness impact, as well as the dynamics of the mutation load is therefore essential for monitoring the health of natural populations.

One system that currently undergoing rapid changes is the Antarctic ecosystem.

Against the backdrop of historical exploitation through sealing and whaling, which massively restructured the krill-dependent food web of the Southern Ocean, climate change now disturbs key ecological parameters through rapid warming and shifting of sea ice coverage and ocean currents.

This also alters the distribution of Antarctic krill, which is furthermore becoming the target of an increasing fishing industry.

A major player in the krill dependent food web is the Antarctic fur seal (*Arctocephalus gazella*), which serves as an indicator species for the Southern Ocean ecosystem.

The fur seals have a very dynamic demographic history, characterized by massive hunting pressures throughout the 19th century.

Within little more than one hundred years upon the discovery of their breeding colonies by James Cook in 1775, this historically abundant species was driven into economical extinction.

Yet, throughout the twentieth century, the Antarctic fur seal experienced a spectacular recovery with millions of animals by the 1970s, even exceeding pre-sealing population size estimates.

More recently, the trend has shifted once more as numbers are declining since the 1990s - this has been linked to changes in the ecosystem, that increasingly cause inbreeding depression in this species.

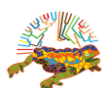
Here, we leverage whole genome resequencing data to estimate the degree of mutation load within the Antarctic fur seal.

We assess the traces of a dynamic demographic history on a species' load, and investigate its impact on current day fitness.

Harnessing a new Hi-C based reference genome, as well as a multi-species genome of alignment of 67 carnivora reference genomes, we combine load estimates inferred from functional effect of mutations with evolutionary conservation based load scores.

The fur seal's mutation load is put into the context of runs of homozygosity (ROHs) that link the load to inbreeding at different time points in an individual's ancestry.

By partitioning the load according to its harboring ROH size class, we investigate the effect of the fur seal's recent demographic history on the processes of purging and drift and how these historical factors continue to shape the fitness of the current population.



Early-life social stress rewrites strategic seminal fluid investment in *Drosophila* males

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POSTER SESSION 2 - **P02.346**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Climate change is intensifying environmental stressors including habitat fragmentation, and resource limitation—all of which can affect developmental conditions with cascading effects on adult reproductive fitness. This can be particularly important for organisms that rely on ephemeral resources that can be easily overexploited, such as, for instance, insect larvae of many fruit fly species. Yet, the mechanistic links between early-life stress in competitive environments and adult reproductive investment remain underexplored, especially in the context of plastic responses in gamete and ejaculate traits.

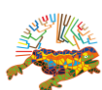
In this talk, we discuss how ecologically relevant developmental stress—larval crowding—affects the production and transfer of seminal fluid proteins (Sfps) in *Drosophila melanogaster*, key molecular drivers of male fertility. Using a fully factorial design and quantitative proteomics, we manipulated larval densities to produce adults of distinct body sizes and tracked how male and female developmental environments shape seminal proteome allocation during mating.

We found that although large males (from low-density larval environments) produced slightly more Sfps overall, small males (from high-density environments) transferred

significantly greater quantities of their seminal proteome per mating. Crucially, small males also increased the transfer of several functionally relevant Sfps, suggesting strategic ejaculate investment shaped by early-life experience. Thus, the effects of crowding were not uniform across all proteins—some Sfps showed targeted, adaptive allocation—suggesting that ejaculate composition itself is a dynamic trait subject to environmental modulation.

Our results highlight an important but underappreciated axis of plasticity: how males allocate ejaculate resources in response to developmental cues indicative of population density and resource stress. In a changing climate, where environmental unpredictability and crowding are likely to become more common, such plasticity could have profound implications for reproductive success, sperm competition, and ultimately, population viability.

Our findings provide mechanistic insight into how climate-linked developmental stress can shape reproductive investment through strategic Sfps allocation. This work advances our understanding of condition-dependent sexual selection and highlights the importance of integrating molecular, ecological, and evolutionary perspectives to predict reproductive responses in a rapidly changing world.



Environmental drivers of parental care diversity in mammals

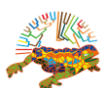
Narzissenfeld N¹, Capellini I¹

¹Queen's University Belfast

POSTER SESSION 2 - P02.373, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Parental care - defined as any parental trait that enhances the fitness of offspring, frequently comes at a cost to future reproduction and survival, thus it should only evolve when the benefits to carers outweigh the costs. Environmental conditions should play an important role in parental care evolution as they modulate pressures like resource availability and predation risk, thus influencing the costs and benefits of care. Care is more likely to evolve under intense resource competition and when environmental conditions are harsh, as under these conditions the benefits of care are likely to increase. In mammals, male care has evolved numerous times and occurs in ~10% of species, but the environmental drivers of male care are poorly understood. Mammals that have allocare – care from non-breeding helpers (i.e. cooperative breeding) –

live in arid environments with less rainfall than socially monogamous species lacking allocare. Here we ask whether male care also evolved in similar environments and is the driver for the evolution of allocare. We address this question while considering also female-only care as well as biparental care and allocare and uncover the specific environmental drivers causing diversity in who cares in mammals. We conducted a phylogenetic comparative study on >500 mammal species to test the associations between care provider, care traits and environmental conditions within the species range. Our results show how arid and unpredictable environments have driven care diversity in mammals, with potential implications for the impacts of climate change on reproduction and animal behaviour.



Environmental O₂ and the *C. elegans* germline

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POSTER SESSION 2 - **P02.368**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Stem cell maintenance and its association to oxygen (O₂) availability is a central focus of our research. While low tissue O₂ levels are known to support the maintenance of pluripotent stem cells in mammals, much less is understood about the role of O₂ in invertebrate stem cell biology. To investigate this, we use *Caenorhabditis elegans* — a soil-living nematode and established model organism — which displays a unique tolerance to a wide range of environmental O₂ levels (spanning almost the full range of 0–100% O₂). Despite this tolerance, adult *C. elegans* worms exhibit a behavioral preference for moderately low O₂ environments (5–12%). We hypothesize that this preference may, in part, reflect that the worms seek out environments of different O₂ levels to adjust internal tissue gradients and cell fates. In *C. elegans*, the only true stem cells are located in the germline, and we are currently exploring two questions: a) can we use the *C. elegans* germline as a model system to study the role of O₂ in stem cell maintenance, and b) how do varying environmental O₂ levels, particularly those above critically low thresholds, influence germline stem cell maintenance and reproductive function in *C. elegans*?

Materials and methods: To explore whether moderately low external O₂ concentrations

improve *C. elegans* stem cell number and fertility compared to standard laboratory conditions, we culture worms under low (2–5%) and high (21%) O₂ concentrations from the L4 developmental stage, using custom-built “hypoxia” boxes. We then compare brood size, hatching rates, and the number of unfertilized oocytes expelled for worms cultured under the different O₂ conditions. To complement the reproduction assays, we are also comparing counts of germline Progenitor Zone cells between O₂ levels, as well as counts of apoptotic bodies in the germline.

Results: Our preliminary results suggest that moderately low (5%) O₂ concentrations do impact the reproductive system in *C. elegans*. Specifically, we see a trend of altered reproductive output and a change in the number of unfertilized oocytes expelled under 21% compared to 5% O₂. The distal region of the germline, however, does not appear to be affected when culturing L4s under 5% O₂ compared to 21%.

Conclusions: Global changes in temperature and precipitation are likely to impact O₂ profiles in soil and water. Using *C. elegans* as a model organism for invertebrate stem cell biology, we hope to gain understanding of how locally changing O₂ concentrations might impact invertebrate reproduction.



Evolution of phenotypic plasticity during environmental fluctuations

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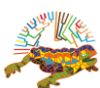
POSTER SESSION 2 - P02.369, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Evolution in fluctuating environments is predicted to disfavor genetic canalization and instead select for alternative strategies, such as phenotypic plasticity or possibly bet-hedging, depending on the accuracy of environmental cues and type of fluctuations. While these two alternatives are often contrasted in theoretical studies, their evolution are seldom studied together in empirical work.

We used experimental evolution for 30 generations in the nematode worm *Caenorhabditis remanei* to simultaneously study the evolution of plasticity and bet-hedging in environments differing only in their temperature variability, where one regime is exposed to faster temperature cycles between 20°C and 25°C, with little autocorrelation between parent and offspring environment, while the other regime had slowly increasing temperature with high autocorrelation in temperature between parent and offspring. These two

environments had the same average temperature over evolutionary time, but one fluctuated on a shorter time scale. After experimental evolution, we scored adult size and fitness in full siblings reared in two different temperatures, optimal 20°C and mildly stressful 25°C.

Experimental evolution in the fluctuating environment resulted in the evolution of increased body size plasticity but not increased bet-hedging, compared to evolution in the slowly changing environment. Plasticity followed the temperature size rule as size decreased with increasing temperature and this plastic response was adaptive since the body size resulting in highest fitness was smaller in the warm temperature. In addition, we documented substantial standing genetic variation in body size, which represents a potential for further evolutionary change.



Genomic signatures of reproductive fitness in the King penguin across two decades of climate change

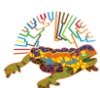
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POSTER SESSION 2 - **P02.370**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Across the millions of genetic polymorphisms that reside within most species, our understanding of their connection to reproductive fitness remains largely limited, especially in wild long-lived vertebrates. Leveraging life-history data from a long-term monitoring program, a newly generated chromosome-level genome assembly, and whole-genome re-sequencing and epigenetic data, we are using the King penguin (*Aptenodytes patagonicus*) as a wild, long-lived vertebrate model to explore the genomic determinants of reproductive fitness. Based on a breeding quality index (BQI), we categorised male and female wild King penguins (≥ 13 years) into two reproductive fitness groups: 1) individuals with high reproductive success ($n = 32$), i.e., individuals which have successfully fledged chicks throughout their lifetime, and; 2) individuals with low reproductive success (n

$= 32$), i.e., individuals which have continually failed at fledging chicks throughout their lifetime. We hypothesised that by accounting for the survival component of fitness (by choosing only long-lived individuals), we will be able to identify the role of genetic diversity and genetic load in determining reproductive fitness. Additionally, by examining high and low fitness individuals sampled across three timepoints (2001, 2008, 2020) ($n = 84$), spanning two decades of Subantarctic temperature fluctuations, we are employing temporal genomics to identify allele frequency shifts in genomic regions associated with reproductive fitness. This research will provide insights into the genetic basis of reproductive fitness and how this crucial component of evolution is being impacted by climate change, elucidating the evolutionary processes that shape resilience in a wild long-lived vertebrate.



How does turbidity and male harassment mediate the female reproductive behaviour and stress?

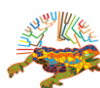
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POSTER SESSION 2 - P02.362, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Due to anthropogenic activities, high turbidity of water has become a frequent occurrence in many waterways. Fish rely on vision for social learning, shoaling, foraging, predator avoidance and mate recognition. But elevated turbidity can limit what they can see, which could potentially disrupt their social dynamics, reproductive interaction and be stressful. Additionally, male sexual harassment, which is characterized by aggressive or persistent approaches by males towards females, could elevate the stress level of females and negatively impact their reproductive success. We assumed that male harassment would be higher in clear water. To investigate the combined effects of turbidity and sexual harassment, we examined the behaviour and cortisol levels (a hormonal measure of stress) of female mosquitofish (*Gambusia holbrooki*).

In a 2x2 experiment, females were placed with either one or three males and in either clear or turbid water. The males were surgically manipulated so that females could not be mated and inseminated by males but could still be harassed. Females in clear water spent significantly less time being chased by males and males made fewer mating attempts towards them than for females in turbid water. And females with one male spent less time being chased by males and males made fewer mating attempts towards them than female with three males. Crucially, female in clear water had significantly higher cortisol levels than those in turbid water, but there was no effect of the number of males. These results indicate that water turbidity rather than sexual harassment is a major source of stress for females.



How thermal stress alters reproductive traits and foraging behavior in Trinidadian Guppies

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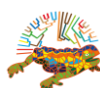
POSTER SESSION 2 - **P02.371**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

With fluctuating temperatures and extreme weather events becoming more common due to climate changes, species must quickly adapt or decline. Increased temperatures affect functions such as foraging, reproduction, and growth, but little is known about these when faced with thermal stress at different stages of life.

Urban aquatic ectotherms are especially vulnerable as they (i) rely on behavioral and physiological mechanisms to thermoregulate, (ii) are restricted on where

they can disperse, and (iii) endure additional heat stress due to the “urban heat island” effect. Despite this, urban areas are hotspots for the establishment of invasive species.

To better understand how species are affected by increasing temperatures, I use an invasive freshwater fish, the Trinidadian Guppy (*Poecilia reticulata*) from urban and less urban populations to examine reproductive traits and foraging behavior in response to heatwaves at different life stages.



Hybridisation and Climate Change in Alpine Orchids

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POSTER SESSION 2 - **P02.351**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

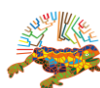
Mountain ecosystems usually contain an elevated level of species richness and endemism. These mountain ecosystems are fragile and are under threat from a range of global change pressures, the most prevalent being climate warming. There has been a well-documented trend of species range shifts and phenological (life history event) shifts in response to climate change, either to higher latitudes or to higher elevations to track isotherms; although not all species follow this trend. This could potentially lead to a disruption of ecosystem networks as range shifts are likely to be idiosyncratic across species, reshaping ecological communities, removing existing biotic interactions, and introducing novel ones, including in the European Alps.

Meadows on different mountains along the same latitude can provide a 'natural laboratory' for examining response to environmental change in a naturally replicated system. Alpine meadows host a variety of plant species including members of the diverse flowering plant family Orchidaceae (orchids). This study will focus on the orchid species *Gymnadenia conopsea*, which occurs at 0 – 2400m elevation and produces a single inflorescence of 10-100 lilac flowers with a long, narrow nectar spur, and *Gymnadenia rhellicani*, which occurs at 1000 – 2800m elevation and has densely clustered dark inflorescences ranging in colour from whitish-pink to very dark red and a short nectar spur. These species occur in sympatry in Alpine meadows and produce F1 hybrids in limited frequencies (less than 1% of the population).

This study will look at the barriers maintaining reproductive isolation between

Gymnadenia conopsea and *Gymnadenia rhellicani* whilst allowing limited hybridisation, and how these differ between field sites because of local adaptation. This will include collecting data on phenology, pollination, and genetics, as well as projecting potential suitable environmental niches of parent species and their hybrid under different future climate scenarios to see the possible impacts of climate warming. Preliminary climate projection models show reduced suitability for *Gymnadenia conopsea* in Italy, the Balkans, and along the border of France and Spain, range contractions for *Gymnadenia rhellicani* across the Alps, and a slight increase in range for their hybrid. As hybrids have only been found as F1s, it is unlikely the range of the hybrids will increase unless the overlap zone of the parent species increases.

Environmental change has the potential to cause ecological disturbance through shifts in species ranges and phenology, and changing biotic interactions, with previously isolated plant lineages establishing secondary contact, increasing potential for hybridisation. The consequences of ecological disturbance are unpredictable, but hybridisation and introgression can lead to the acquisition of favourable alleles which may be beneficial when species are facing rapid environmental change. The *Gymnadenia* orchids and their hybrids are a good model study system for inter-species interactions under rapidly changing environmental conditions. Determining how species are being affected by environmental change could lead to more informed and targeted conservation efforts for species in mountain ecosystems.



Impacts of thermal and salinity stress on flatworm reproduction: sex-specificity, genetic variation and mechanisms

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POSTER SESSION 2 - **P02.363**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Thermal fertility limits have recently emerged as a key aspect of animal biology for understanding the likely impact of climate change. However, in nature, heat stress will often combine with that from other environmental parameters, meaning that we ultimately need to investigate multivariate responses to predict evolutionary outcomes. Here we present a series of experiments in the marine flatworm *Macrostomum lignano* designed to clarify the short-term impacts of stress induced by both temperature and salinity change in order to quantify the extent to which these environmental factors might combine to suppress reproduction under future climate change scenarios. Specifically, we aim to determine the relative

contributions to infertility through male and female reproduction; to estimate the separate and combined effects of chronic and acute thermal stress regimes, and the separate and interactive effects of thermal and salinity stress (which in nature will often co-occur); to test for genetic variation in all of these responses indicating the potential for them to evolve; and to identify the underlying traits responsible for mediating the reproductive outcomes. Coupled with experimental evolution approaches currently underway to understand long-term responses, these results establish *M. lignano* as a useful model system to study climate change impacts on animal fertility.



Is the incubation stage a bottleneck for avian fitness in a warming world? Evidence from an experimental study on collared flycatchers.

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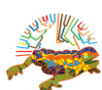
POSTER SESSION 2 - P02.366, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Understanding how temperature influences avian reproductive success requires detailed knowledge of its effects at the level of the nesting environment. In our research, we examined how variation in ambient temperature within nests affects offspring fitness-related traits in a population of migratory collared flycatchers (*Ficedula albicollis*) on Gotland Island. Over three breeding seasons, we experimentally manipulated nest temperatures using four treatments: heating during the incubation period only, heating during the nestling period only, heating during both incubation and nestling periods, and an unmanipulated control. This experimental design allowed us to assess how elevated temperatures during different developmental stages influence offspring performance.

Our results showed that only heating during the incubation period significantly affected

traits linked to offspring fitness. In contrast, heating during the nestling stage, or throughout the entire nesting period, had no detectable effect, and there was no interaction between these two periods in our experiment. These findings suggest that temperature conditions during embryonic development may play a particularly important role in shaping early-life traits in passerines—not necessarily through direct effects on the developing embryos, but potentially indirectly via their influence on female behaviour and incubation patterns.

By examining temperature variation at the scale of individual nests, our study provides new insights into how local environmental conditions may shape early developmental stages in birds. The observed effects, however, may reflect population-specific responses shaped by the local ecological context.



Loss of Photoperiodic Regulation of a Key Pleiotropic Gene Underlying the Evolutionary Diversification of Reproductive Seasonality in Sticklebacks

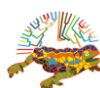
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POSTER SESSION 2 - **P02.352**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Understanding how organisms adapt their reproductive strategies to changing environmental conditions is essential for predicting population persistence in the context of climate change. In seasonal environments, many species use photoperiod as a reliable cue to synchronize reproduction with optimal environmental conditions. However, climate change can decouple photoperiods from actual seasonal resource availability, leading to phenological mismatches that compromise reproductive success. Despite its ecological importance, the molecular and genetic mechanisms underlying variation in reproductive timing remain poorly understood. Here, we investigate the genetic and evolutionary mechanisms underlying interpopulation divergence in reproductive seasonality in the three-spined stickleback (*Gasterosteus aculeatus*), a species showing dramatic divergence in reproductive timing between marine and freshwater ecotypes. Ancestral anadromous marine ecotypes reproduce seasonally in response to increasing day length, derived freshwater ecotypes, particularly stream ecotypes in lower latitudes, have evolved extended or nearly continuous reproductive periods throughout the year. We found that testis development in marine ecotypes is stimulated by a shift to a long photoperiod, whereas stream ecotypes that evolved independently in Japan and North America have lost this photoperiodic response. Transcriptomic analyses of the pituitary

gland revealed that expression of thyroid stimulating hormone beta 2 (TSH β 2) gene responds to photoperiod in marine ecotypes, but not in stream ecotypes. Functional validation using TSH β 2 knockout in marine ecotypes induced reproductive traits—including testis development and courtship behavior—even under short-day conditions that normally suppress reproduction. These knockout fish also resembled stream ecotypes in brain transcriptome profiles, body size, and hormone levels, indicating a pleiotropic role of TSH β 2 in life-history regulation. Using single-cell transcriptomic and epigenomic profiling, we identified a novel pituitary cell cluster that exclusively expresses TSH β 2. Under short-day conditions, this cluster exhibited increased TSH β 2 expression and enhanced chromatin accessibility in its upstream region, suggesting transcriptional and epigenetic regulation in response to photoperiod. Furthermore, linkage mapping revealed that convergent loss of TSH β 2 regulation in stream ecotypes has occurred through distinct genetic mechanisms—cis-regulatory mutations in North America and trans-regulatory changes in Japan. Together, these findings demonstrate how regulatory changes in a single gene can mediate adaptive or maladaptive shifts in reproductive seasonality under altered seasonal environments, highlighting potential consequences for population persistence under ongoing climate change.



Masting modeling: evolution towards an early phenology to control seed consumers.

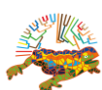
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POSTER SESSION 2 - P02.367, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

In many species of perennial plants, the intensity of fruiting is extremely variable from one year to the next and synchronized at the population level. This reproductive strategy, known as 'masting', has major ecological and economic repercussions (notably the masting of oaks) and also represents an evolutionary enigma. Based on a theoretical approach, we test the hypothesis that the evolution of floral phenology could play a pivotal role in controlling masting. A relatively early phenology would often expose the trees to climatic contingencies (late frosts), leading to frequent reproductive failures and occasionally, great fruiting success. Thanks to the consumer starvation-satiation mechanism, the resulting stochastic fruiting dynamics would be particularly effective in controlling a wide diversity of consumers, such as the insects that parasitise oak acorns. Trees could thus save a large proportion of their seeds from insect infestation. To assess the relevance of this hypothesis, an individual-based and stochastic eco-evolutionary model was developed, coupling a forest regeneration model with models of tree reproduction dynamics, floral phenology and insect

community dynamics. In these models, individuals with different phenological strategies are introduced and compete for space (replacing dead adult trees) through recruitment from insect-free seeds. Using the adaptive dynamics framework, we show that insect selection pressure can make the phenology evolve towards greater precocity, generating stochastic fruiting dynamics and thus efficiently controlling insect populations. Also, we situate our study within the framework of the tragedy of the commons integrating an insect dispersion model. We show these results are conditioned by a privatization of the tree's insect pool, meaning that individuals can save their own seeds by starving "their" local consumers whereas a full dispersion of insects dilute the benefits of an invasive masting strategy across all trees. Our research demonstrates that early phenology, despite frequent reproductive failures, can provide a selective advantage, contrary to conventional intuition. As phenology is very sensitive to climate change, we can expect major changes in the masting of oak trees, with far-reaching repercussions on the dynamics of the associated ecosystems.



Mitonuclear interactions constrain thermal responses

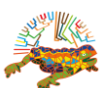
Camus F¹

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POSTER SESSION 2 - **P02.372**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Metabolism lies at the core of life-history theory, and the ability to adapt to fluctuating environmental conditions is key for survival and fitness. Temperature is one of the most pervasive ecological stressors, influencing metabolic rate, development, and energy allocation. Mitochondria play a central role in temperature adaptation, as they regulate both energy transduction and intermediary metabolism, processes that directly impact growth and reproductive success. However, oxidative phosphorylation (OXPHOS) is uniquely vulnerable to disruption, as it relies on gene products encoded by two separate genomes—mitochondrial (mt) and nuclear (n)—which must remain functionally coadapted despite their distinct evolutionary pressures. Temperature fluctuations pose a unique challenge to this mitonuclear balance, as shifts in metabolic demand can exacerbate intergenomic incompatibilities. Here, I provide evidence that mitonuclear interactions influence resource allocation and metabolic efficiency in response to temperature variation, affecting growth and development in *Drosophila melanogaster* natural populations. Using a panel of coevolved and admixed mitonuclear

genotypes, we demonstrate that temperature stress can amplify the physiological consequences of mitonuclear mismatches, leading to delayed development, altered metabolic efficiency, and reduced fitness. To gain a mechanistic understanding of these effects, I will also present RNA-Seq data exploring how mitonuclear coexpression changes in response to thermal stress. By linking genomic regulation with physiological traits, these data will reveal the molecular pathways underpinning temperature-driven shifts in metabolism and development. Results indicate that intergenomic epistasis has cascading effects across biological hierarchies, from cellular bioenergetics to organismal performance, and that ecological stressors stemming from a rapidly changing climate could intensify these incompatibilities, increasing the risk of fitness breakdown in hybrid populations. Understanding how mitonuclear interactions shape temperature adaptation is essential for predicting evolutionary responses to climate change and identifying key genetic pathways that buffer organisms against metabolic stress.



Phenological fluctuations but no advance: exploring the drivers and consequences of breeding phenology in the Soay sheep

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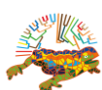
POSTER SESSION 2 - **P02.350**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The annual patterns of life history traits, or phenology, are often affected by environmental conditions, with different conditions acting either as a cue or as a constraint for biological processes. Phenological changes in response to climate change are well-documented across avian, plant, and insect systems, with many studies reporting strong shifts in the timing of life-history events. Such phenological shifts can have important consequences for an individual's growth, survival, and overall fitness. However, the extent to which mammalian phenology is sensitive to climate and the degree to which phenology matters for mammalian fitness is lacking. To address this gap, we first examine the sensitivity of Soay sheep birth dates to weather variables and secondly explore the impact of an individual's birth date on its survival.

We use data from a long-term, individual-based study system of Soay sheep on St Kilda, Scotland, with 5646 known birth dates spanning over three decades. Individuals are tagged and followed throughout their life to obtain genetic, morphometric, life-history, and behavioural data. Environmental data on daily temperature, wind, and rain is available across the study period.

We find little evidence for a trend in birth date of Soay sheep from 1987 – 2023, despite local weather showing average warming of 0.5°C over this period. Mean annual birth dates fluctuate across time, but we found little evidence that weather during gestation shapes this variation. We found that birth date predicts lamb first-year survival beyond previously established factors (e.g., birth weight, population density and winter weather), with some evidence of stabilising selection toward an intermediate optimum birth date for survival that varies between years.

The absence of a phenological trend over time in Soay sheep differs from many other temperate study systems, which often show an advance in reproductive phenology in the spring in response to warming conditions. However, our analyses of lamb survival demonstrate the phenology does matter ecologically in this system and suggests that optimising selection may be operating on the timing of breeding in this population. This work further emphasises the importance of developing and maintaining long-term study systems, particularly in large mammals, which can show responses to climate change differing from other, more well-studied taxa.



Phenotypic Selection on Reproductive Traits Under Autonomous Self-Fertilization in *Fragaria vesca*

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POSTER SESSION 2 - **P02.341**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

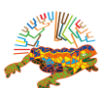
Understanding how reproductive traits interact to shape fitness is fundamental to deciphering the evolutionary dynamics of plant mating systems. In *Fragaria vesca* (wild strawberry), floral traits such as petal size, herkogamy (spatial separation of anthers and stigmas), and inflorescence exsertion influence the balance between selfing and outcrossing. While individual reproductive traits have been extensively studied, the role of multi-trait interactions in determining selfing efficiency remains unclear, particularly under conditions of autonomous self-fertilization—where pollinators are excluded. Additionally, environmental variables such as temperature, precipitation, and light availability may further shape floral trait expression and selection pressures, making it imperative to understand how ecological conditions mediate reproductive strategies.

In this study, we investigate how interactions among reproductive traits influence selfing efficiency and fitness outcomes in *Fragaria vesca* by integrating morphological, ecological, and evolutionary perspectives. We analyze key floral traits, including petal size, lateral and vertical herkogamy, inflorescence exsertion, flower number, and flower volume, assessing their combined effects on fruit set, fruit volume, and the proportion of fertilized achenes—critical

indicators of reproductive success. By experimentally excluding pollinators, we isolate the effects of floral morphology on autonomous selfing efficiency, providing a controlled framework for examining selection pressures on reproductive traits.

To quantify phenotypic selection, we employ multivariate statistical analyses and linear mixed models (LMMs), enabling us to disentangle trait correlations and assess multi-trait selection gradients. Furthermore, we incorporate environmental data to evaluate how variation in temperature, precipitation, and light availability influences both trait expression and selection dynamics. This approach allows us to explore genotype-by-environment interactions, shedding light on how selective pressures on reproductive traits shift across ecological contexts.

By elucidating the interplay between reproductive traits and environmental variation, this study provides novel insights into the adaptive mechanisms shaping selfing efficiency in *Fragaria vesca*. Our findings contribute to a broader understanding of how floral traits evolve in response to selection under selfing conditions, with implications for predicting plant reproductive strategies in the face of environmental change.



Predicting reproductive fitness under climate change: developmental effects of nutritional and thermal stress in locally-adapted populations of *Drosophila melanogaster*

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POSTER SESSION 2 - **P02.347**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

As the climate changes, populations must overcome more frequent and extreme exposures to a wide range of stressors. However, our knowledge of how locally-adapted populations respond to combinations of stressors, particularly during development, remains incomplete. Recent studies show that elevated temperatures can interact with nutrition to accentuate the negative effects of a poor diet, suggesting higher developmental costs of nutritional stress when individuals experience temperatures outside of their locally-adapted conditions. Here, we used the Geometric Framework for Nutrition to test this hypothesis using two locally-adapted populations of *Drosophila melanogaster* from opposing ends of a well-characterised adaptive gradient along the east coast of Australia (tropical vs.

temperate). We found that the negative effects of nutritional stress on viability and final body size were significantly greater in the tropical population under warmer temperatures. In contrast, the temperate population was able to utilise a broader nutritional space to maintain high viability and a large final body size across the range of fluctuating temperatures. Our findings reveal how local adaptation shapes the way populations navigate the nutritional space during development in response to increasingly stressful thermal conditions. Importantly, these results suggest that populations adapted to environments with greater thermal and nutritional variability may be better able to maintain reproductive fitness under climate change, while those from more stable environments could face a higher risk of local extinctions.



Reproductive plasticity in response to short-term polystyrene nanoplastic exposure in aquatic ecosystems

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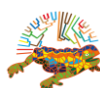
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POSTER SESSION 2 - P02.357, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Species evolution has always been described as slow and long-term processes in which plasticity plays a key role. But how are species facing evolution and adaptation in rapidly changing environmental conditions? Nanoplastics pollution is a rising environmental concern whose impacts on biodiversity and human health are far from being understood. This is particularly salient in aquatic ecosystems, where most vertebrate species depend on external fertilization for reproduction. Here we evaluated the effects of a short-term exposure to polystyrene nanoplastics (NPs) in the zebrafish germline to further explore their direct impact on reproduction and its evolutionary plasticity. To this end, zebrafish (*Danio rerio*) were exposed to 5 mg/L of 45 nm polystyrene (PS)-NPs via water for 96 h. We show that in males, nanoplastics induced testicular histological alterations

with abnormal sperm clustering and chromatin compaction, resulting in viable spermatozoa but with reduced motility. Moreover, in females we observed an alteration in oocyte stages frequencies during oogenesis, possibly reflecting alterations in oocyte growth. RNA-sequencing analysis in male testis links nanoplastic induced alterations in the expression of genes involved in chromatin structure, meiosis and DNA double-strand break formation and repair progression, and gametes recognition. Overall, our results demonstrate that even if an NPs acute exposure can be greatly and directly compromising reproductive fitness, species are rapidly adapting and developing mechanisms to ensure their maintenance and persistence.



Resource availability as a key environmental constraint on maturation in the ectothermic vertebrate rainbow trout (*Oncorhynchus mykiss*)

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POSTER SESSION 2 - P02.358, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Knowledge of how climate-driven environmental change impacts reproductive success is crucial for understanding species evolution in a rapidly changing world. While model organisms have elucidated many aspects of reproduction physiology and behavior, salmonid fishes like rainbow trout (*Oncorhynchus mykiss*) offer powerful and ecologically relevant insights into how environmental changes may interact with vertebrate life-history strategies. Rainbow trout, as other ectotherms, face an important constraint in warming waters since higher temperatures increase metabolic demands but often reduce resource availability. To understand how food availability influences maturation rate in rainbow trout, we conducted a common-garden experiment with approximately 3000 individuals from five full-sibling families. Fish were maintained from fertilization for two years in two different environmental conditions, a control group fed at standard levels and a food-restricted group receiving only 66% of the normal ration. All individuals were measured at three-month intervals throughout the experiment, with both length and weight recorded at each time point, along with assessments of maturation status. Our results showed that fish reared under food-restricted conditions had significantly reduced weight and length across all families, reflected in a lower body condition. This reduction reflects the limited energy reserves available for growth, directly resulting from reduced resource availability, highlighting this species' limited capacity for compensatory feeding and plasticity under prolonged nutritional reduction. After the two years, the expected timeframe for initial maturation in this population, maturation was observed in

much higher rates in the control treatment and only among males. No females matured in either treatment indicating the substantial increase in energy demand for female reproduction, which might impose even stronger sex-specific constraints on reproductive timing under nutritional reduction. Furthermore, a more detailed phenotypic analysis using dual-energy X-ray absorptiometry (DEXA) imaging revealed substantial differences in body composition between treatments, specifically in fat-to-lean mass ratios. These findings further support the conclusion that individuals in prolonged nutritional restriction more often lacked the fat reserves necessary to surpass the physiological threshold required for maturation, a delay that can significantly reshape the evolutionary trajectory of a population. This work underscores the ecological and evolutionary relevance of reduced resource availability in a warming world, where the metabolic needs for maturation are most likely not matched by the necessary resources. Importantly, our design allowed us to disentangle genetic and environmental contributions to maturation, revealing consistent family-level responses that hint at heritable variation in reproductive plasticity under lower nutrition. Overall, this study highlights how nutritional restriction can reshape reproductive patterns through energetics, with potentially profound implications for population dynamics and life history strategies in warming climates. It also demonstrates the value of salmonids as a robust and informative, yet still underutilised, non-model system for investigating vertebrate reproduction in experimental eco-evolutionary research.



ShareTrait: towards interoperable and reusable trait data in Ectotherms

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POSTER SESSION 2 - P02.353, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

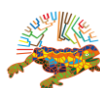
Data on species traits are increasingly being synthesised to comprehend how species respond to and affect their environment, and how trait adaptations have evolved to explain extant trait variation. Effective use and synthesis of trait data studies is hampered by inadequate standards for sharing and re-using data across studies, including the associated metadata. ShareTrait is a novel initiative focused on compiling and standardising individual-level trait data in ectotherms. It is structured as a relational database, allowing access to trait data and associated metadata in an interoperable and reusable format. The first proof of concept focused on three key traits that are comparable across ectotherms: metabolic rate, development time, and fecundity.

ShareTrait is now expanding the range of ectotherm traits and associated metadata, including those informative at higher levels of biological organisation (e.g., replicate groups, populations). To support this, there is an ongoing collaboration with the European Thermal Fertility Network (an ESEB STN), which advocates for the collection of standardised experimental and meta-analytical data on thermal limits to survival and fertility. As a result, new trait data are being incorporated on survival, longevity, and fertility (measured either as group-level outcomes or through proxies such as gametic and gonadal traits) in response to environmental stressors. Individual-level variation is preferred, as it represents the standing variation upon which natural selection acts. However, these data may be informative at levels beyond the individual, forming the foundation for integrating traits

best measured at the population level but often recorded as binary outcomes at the individual level, such as absolute fertility, survival, and developmental viability.

To enhance cross-taxon comparability and facilitate comparative evolutionary and ecological analyses, ShareTrait incorporates a built-in pipeline generating a tree of the species included pruned from the Open Tree of Life. Towards the same goal, a standardised life-stage classification based on Dynamic Energy Budget (DEB) theory is being developed to be embedded within the relational database structure.

Designed as a tool for comparative trait-based research, ShareTrait offers standardised data integration, rich metadata, citation and attribution guidance, and analytical recommendations for effective data reuse. With ongoing collaborations such as the one with the European Thermal Fertility Network, the range of traits, trait types, and metadata is expanding—allowing researchers to share data in an interoperable and reusable format. The high data resolution, methodological transparency, and analytical flexibility that this initiative promotes make it a key resource for advancing the implementation of FAIR principles for animal trait data. As the platform continues to grow to include a wider array of traits, data submission options will expand in the coming years, streamlining research efforts and empowering researchers to explore broader ecological, evolutionary, and physiological questions.



Stage-Specific Nutritional Environments Drive Plastic and Evolutionary Shifts in Reproduction

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POSTER SESSION 2 - **P02.345**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

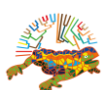
Background: Reproduction is highly sensitive to environmental changes, and climate-induced shifts in food quality and availability are emerging as critical challenges across taxa. While the effects of caloric intake on reproduction are well-documented, the ecological and evolutionary consequences of dietary macronutrient composition on reproductive timing and ageing remain poorly understood. Moreover, how dietary variation across life stages shapes age-specific reproductive plasticity and drives long-term evolutionary responses under multigenerational environmental change remains largely unexplored.

Materials and Methods: We used a large, genetically diverse population of *Drosophila melanogaster* ($n \approx 2100$) to examine the effects of isocaloric larval and adult diets differing in P:C ratio on female reproductive ageing. We quantified age-specific fecundity, ovariole number, and vitellogenesis, and performed RNA-seq on flies exposed to a fully factorial design of larval–adult dietary combinations. To assess evolutionary responses, we conducted a long-term selection experiment over 70 generations under these four stage-specific dietary regimes, measuring shifts in reproductive timing and investment.

Results: We observed pronounced age-dependent effects of diet composition on reproductive output: early-life fecundity was primarily influenced by larval diet, mid-life by both larval and adult diets, and late-life by adult diet alone. These effects were additive,

with no significant interaction between the two life stages. Mechanistically, larval diet impacted reproduction through both ovariole number and vitellogenesis, while adult diet modulated reproduction primarily via vitellogenesis. RNA-seq analyses revealed robust gene expression plasticity, with distinct age- and diet-specific transcriptional signatures in pathways related to metabolism, reproduction, and nutrient sensing. Notably, populations evolved under carbohydrate-rich larval diets exhibited a pronounced shift toward earlier reproductive investment, indicating an evolved response distinct from short-term diet-induced plasticity.

Conclusions: This study demonstrates how stage-specific macronutrient environments—paralleling those increasingly encountered under climate change—modulate reproductive ageing via distinct physiological and molecular pathways. Furthermore, sustained exposure to these environments can drive evolutionary shifts in reproductive schedules. These findings have broad implications for understanding how species may adjust their reproductive strategies in response to climate-altered nutritional landscapes, highlighting the intertwined roles of plasticity and evolution. By integrating life-history theory, nutritional ecology, and transcriptomic analysis, our work provides a mechanistic and evolutionary framework for predicting reproductive resilience under global environmental change.



Stochastic reproductive plasticity in *Artemia* brine shrimps: bet-hedging against unpredictable environmental change?

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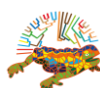
POSTER SESSION 2 - **P02.343**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Life in highly variable environments requires sophisticated mechanisms enabling organisms to cope with challenging living conditions. Phenotypic plasticity – the ability for a same genotype/organism to produce several phenotypes in response to rapid changes in their surrounding conditions – can help organisms mitigate the impact of sudden and unfavourable environmental changes. But what does it mean when phenotypic plasticity occurs in the absence of environmental variability?

The (commercially-important) extremophile *Artemia* brine shrimps (Crustacea, Branchiopoda) have evolved a unique adaptation to survive in variable hypersaline environments. Females reproduce ovoviviparously by giving birth to live offspring when the conditions are favourable to population growth, but can rapidly switch to oviparity, releasing highly resistant diapaused eggs in their environment when conditions become unfavourable. This reproductive shift was long thought to be

exclusively controlled by variations in environmental conditions. While we confirm that *Artemia* females are capable of reproductive plasticity in response to abiotic stimuli, we also report that they keep switching between these two reproductive modes under benign and constant conditions. Our repeated records of stochastic reproductive plasticity in different *Artemia* populations led us to hypothesise that these extremophiles have not just evolved the extraordinary ability of being reproductively plastic in response their environment, they also hedge their bets when it comes to offspring production.

In addition to presenting an extensive dataset of phenotypic records of this phenomenon, we explore what is happening at the gene expression level and we use population demography modelling to determine which environmental scenario could favour the evolution of diversified bet-hedging over phenotypic plasticity alone.



Thermal genotype-by-environment interactions and sexual selection in *Drosophila melanogaster*

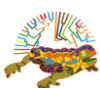
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POSTER SESSION 2 - **P02.342**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Temperature is a salient ecological factor with a strong and ubiquitous influence on the biology of living organisms. Yet, we know little about how the thermal variation to which organisms have adapted to affects sexual selection. Using wild *Drosophila melanogaster*, we studied thermal genotype-by-environment interactions (GxE) in the reproductive success of males and females in competition against rivals for reproduction. We derived isogenic lines from a wild population and set up controlled crosses to construct heterozygous genotypes, and then proceeded to estimate F1 reproductive success in competitive

assays across the thermal range at which reproduction is optimal for this population (20°C, 24°C and 28°C). We found evidence of strong thermal GxE in males (but not females), characterized by quadratic reaction norms indicative of males that were either relatively better competitors at the average (vs. extreme temperatures) or vice versa. Overall, our results join other recent studies in suggesting that temperature modulates sexual selection, and that environmental heterogeneity may foster the maintenance of substantial genetic variation in reproductive success.



To self or to clone? Mechanisms driving reproductive assurance strategies across wild strawberry genotypes.

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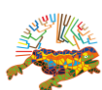
POSTER SESSION 2 - **P02.340**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Under the increasingly frequent pollinator limited environments, plants will need to rely on modes of reproductive assurance such as selfing and cloning. However, few studies look into the interplay between selfing and cloning in plants that can do both. Here, we explore the mechanisms that determine the relative expression of selfing and cloning throughout the European distribution range of the wild woodland strawberry. Specifically, we evaluate whether the allocation to autonomous selfing and cloning under a pollinator free environment is determined by resource competition or by factors limiting autonomous selfing, such as poor anther-stigma contact and early inbreeding depression. We also tested whether the capacity to self is higher closer to pole or at higher altitudes, assuming increased pollination uncertainty in these sites.

Materials and methods: We established an outdoor common garden experiment with 121 wild strawberry genotypes (*Fragaria vesca*) from across Europe and excluded them from pollinators. On each genotype, we recorded several reproductive traits and performed hand pollination treatments.

Results: We found a weak tradeoff between runner production and the quality of autonomous fruits (proportion of fertilized achenes). The capacity to autonomously self-fertilize was determined by the lateral proximity of the anthers to the pistils in the flowers (lateral herkogamy) and somewhat by early inbreeding depression. Contrary to our expectations, genotypes sampled at higher latitudes and altitudes were significantly worse at self-fertilizing. Genotypes at higher latitudes and altitudes had significantly larger flowers.

Conclusions: Both resource competition and factors limiting the capacity to autonomously self-fertilize impact the relative expression of the reproductive assurance modes. The inverse relationship between the capacity to autonomously self-fertilize and petal size at a latitudinal and altitudinal gradient suggests that the pollen limitation likely experienced at higher latitudes and altitudes is selecting for increased outcrossing rather than for increased reproductive assurance through selfing.



What doesn't kill me makes me stronger: How naturally occurring insecticide resistance alleles lead to broader colonising opportunities in *D. melanogaster*

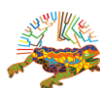
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POSTER SESSION 2 - **P02.344**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Nutrition is essential to all living organisms affecting life-history traits such as longevity, reproduction, and stress resistance. However, the nutritional landscape is a dynamic environment in constant change. A human-made example is the case of agricultural pests and their respective nutritional niches. Not only do crops represent new nutritional opportunities for insects, but the use of insecticides in the last century represents a novel toxic property of this new food source. Additionally, the food animals exploit plays an important role in defining their ecological niche and abundance. To colonise new diets, animals must adapt to the nutritional, physical, and chemical properties of the food. Consequently, insecticide resistance arose as an adaptation to combat the adverse effects of a changing environment in the form of these anthropogenic compounds. Insecticide resistance must, therefore, involve a concurrent adaptation to the novel nutritional niche that harbours the insecticide. We investigated how the presence of a naturally occurring insecticide resistance allele in *D. melanogaster* impacts

life-history traits across different nutritional niches both in the presence, and absence, of insecticides. With the use of the nutritional geometry framework, we compared susceptible and resistant flies and observed how the time of development from egg to adult, and female and male fertility changed across diets differing in their protein to carbohydrate ratio and their caloric content. Our results show that not only do resistant flies develop faster across environments, but also the fertility optima for both sexes changes with just one allelic change. These outcomes suggest future impactful consequences for the evolution of insecticide resistance and how resistant insects may colonise new nutritional niches despite the toxic effects of these xenobiotics. A better understanding of how nutrition impacts the evolution of insecticide resistance will lead to improved knowledge of the evolutionary dynamics of naturally occurring resistance alleles. Together, these insights enlighten us on how adaptation to environmental changes impacts species' persistence and abundance.



Climate change impacts on pup production, survival and recruitment in Antarctic fur seals

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MEETING ROOM 115, August 20, 2025, 10:30 AM - 12:30 PM

Global climate change is known to negatively affect many aspects of natural systems. Nevertheless, we still do not fully understand how individuals are responding to worsening environmental conditions. Interactions between genetic and environmental factors may be important in determining such responses, but little attention has been dedicated to date to this topic, especially because of the technical challenges associated with generating long-term datasets. Here, we use three decades of detailed demographic data collected from an Antarctic fur seal (*Arctocephalus gazella*) colony breeding at Bird Island (South Georgia), coupled with environmental data and detailed measurements of inbreeding, a fundamental metric of individual genetic quality, to uncover the determinants of seasonal reproductive success, a particularly well-suited trait to study individual responses to climate change. By decomposing seasonal reproductive

success in its three main components, pup production, survival and recruitment, we disentangled the effects of various factors affecting reproduction in this species. We found that the climate change induced increase of the Southern Annular mode (SAM) index, which modulates food availability, is negatively associated with all aspects of reproductive success. Moreover, we found evidence for pup production and survival, but not recruitment, being both density- and age- dependent. Finally, we uncovered a significant interaction between inbreeding and SAM index, showing that less inbred individuals can adopt an adaptive response to climate change, as opposed to more inbred individuals. This result highlights the importance of genetic effects in determining individual responses to climate change, which we speculate may be mediated by inherent differences in pace-of-life syndromes between inbred and non-inbred individuals.



Do heatwaves affect insect reproductive behaviour?

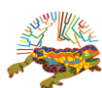
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MEETING ROOM 115, August 20, 2025, 10:30 AM - 12:30 PM

Extreme climatic events, such as heatwaves, are increasing in frequency and intensity under ongoing climate warming, posing a severe threat to biodiversity. The occurrence of unpredictable stressful thermal conditions are particularly disruptive for organisms' biological functions, especially in ectotherms such as insects. Being key for population fitness and persistence, understanding the impact of heatwaves on reproduction is particularly important. Yet, the effects of heatwaves on animal reproductive behaviour remain largely unexplored. This is surprising given that temperature can importantly modulate behavioural interactions among mating partners. We test whether heat stress derived from ecologically relevant heatwaves affects the survival and mating

behaviour of the common house cricket *Acheta domesticus*. We exposed adult males and females to simulated heatwave conditions of different magnitudes (32C and 36C) and durations (3 and 5 consecutive days) and measured their survival, mating behaviour (i.e., male courtship, female mate choice) and mating success when paired with unstressed partners. We expect costs of thermal stress to be revealed by reduced expression of courtship and successful mating, compared to individuals kept at controlled conditions (27C). Our results on the impact of nonlethal heat stress on sex-specific trait expression can provide valuable insights into the long-term effects of heatwaves on insect population decline and biodiversity loss related to climate change.



Environmental Influences on Sexual Selection and Reproductive Trait Divergence in a Sex-Role Reversed Insect

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MEETING ROOM 115, August 21, 2025, 10:30 AM - 12:30 PM

Anthropogenic land use change, a major driver of global biodiversity loss, is increasingly recognised as an important evolutionary force with consequences for reproductive behaviour and sexual selection. As landscapes are transformed through urban development, agriculture, and other human activities—often in concert with climate change—species face novel ecological pressures that can reshape mating systems, reproductive traits, and evolutionary trajectories. However, how these changes influence sexual selection remains poorly understood, particularly in species with unconventional reproductive strategies.

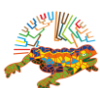
The sex-role reversed dance fly *Rhamphomyia longicauda* provides an opportunity to explore these dynamics. In this species, females compete for access to choosy males who offer costly nuptial gifts—prey items essential for female reproductive success. To attract males, females display conspicuous sex-specific ornaments, including inflatable abdominal sacs and feathery leg bristles. Dance flies are thus an excellent system for investigating how land use and climate change affect the interplay between ecology, behaviour, and reproduction in both sexes.

I examined populations of *R. longicauda* across a land use gradient in southern Ontario, the most populous region of Canada. The study area encompassed urban, suburban, and rural habitats. Specifically, I tested whether patterns of

sexual selection and reproductive trait expression—such as operational sex ratio, ornament size, and nuptial gift diversity—varied with increasing human influence on the landscape.

My findings reveal that land use change significantly influences the dynamics of sexual selection in this species. In warmer temperatures, the operational sex ratio shifted to be more female-biased, and in more urbanised areas, female ornaments were significantly larger than in rural populations. These findings suggest that environmental conditions can strongly affect measurements of sexual selection on females. As expected, we also found a significant decrease in the taxonomic diversity of prey items provided as nuptial gifts in cities, where insect biodiversity is known to be lower.

These results suggest that land use change acts as a selective agent on reproductive traits by altering the costs and benefits of mate acquisition strategies. They also highlight the complex and sometimes counterintuitive ways in which anthropogenic pressures—often intertwined with broader climate-driven habitat shifts—can reshape evolutionary outcomes. For sex-role reversed species like *R. longicauda*, where reproductive success is tightly linked to resource exchange and behavioural signalling, shifts in urban microclimate, prey availability, and rising temperatures may all contribute to the observed changes in selection and reproductive success.



Genotypic responses to different environments and reduced precipitation reveal signals of local adaptation and phenotypic plasticity in woodland strawberry

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MEETING ROOM 115, August 20, 2025, 10:30 AM - 12:30 PM

Background and Aims. Climate change is causing increasing temperatures and drought, creating new environmental conditions, which species must cope with. Plant species can respond to these shifting environments by escaping to more favorable environments, undergoing adaptive evolution, or exhibiting phenotypic plasticity. In this study, we investigate genotype responses to variation in environmental conditions (genotype-by-environment interactions; $G \times E$) over multiple years to gain insights into the plasticity and potential adaptive responses of plants to environmental changes in the face of climate change.

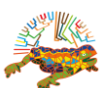
Methods. We reciprocally transplanted 16 European genotypes of *Fragaria vesca* (Rosaceae), the woodland strawberry, between four sites along a latitudinal gradient from 40°N (Spain) to 70°N (northern Finland). We examined $G \times E$ interactions in plant performance traits (fruit and stolon production and rosette size) under ambient weather conditions and a reduced precipitation treatment (as a proxy for drought), at these sites over two years.

Key Results. Our findings reveal signals of local adaptation for fruit production at the latitudinal extremes of *F. vesca* distribution. No clear signals of local adaptation for stolon production were detected. Genotypes from

higher European latitudes were generally smaller than genotypes from lower latitudes across almost all sites, years and both treatments, indicating a strong genetic control of plant size in these genotypes. We found mixed responses to reduced precipitation: while several genotypes exhibited poorer performance under the reduced precipitation treatment across most sites and years, with the effect being most pronounced at the driest site, other genotypes responded to reduced precipitation by increasing fruit and/or stolon production and/or growing larger across most sites and years, particularly at the wettest site.

Conclusions. This study provides insights into the influence of different environments on plant performance at a continental scale. While woodland strawberry seems locally adapted in more extreme environments, reduced precipitation results in winners and losers among its genotypes. This may ultimately reduce genetic variation in the face of increasing drought frequency and severity, with implications for the species' capacity to adapt.

Keywords: Climate change, Drought, *Fragaria vesca*, Local Adaptation, Phenotypic Plasticity, Plant Performance, Reduced precipitation.



Guardians of the germline: small-RNAs and transposable elements impact on Zebrafish fitness in thermal-stressed environments

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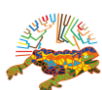
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MEETING ROOM 115, August 21, 2025, 10:30 AM - 12:30 PM

Environmental changes affect organisms from the physiological to the genomic level. Small RNAs (sRNAs) and transposable elements (TEs) can be mobilised through stress, impacting the somatic and germline genome. We assessed the importance of sRNAs and TEs in coping with changes in environmental temperature in the germline of adult zebrafish *Danio rerio*. Understanding this molecular response in the germline helps to understand whether any effects due to temperature are potentially adaptive and truly non-genetic, or whether they have a genomic underpinning in the form of TE activity. FishPi- a piRNA:TE target prediction tool to link differentially expressed piRNAs and TEs, and FishTEA – to link differentially expressed genes and TEs were developed and used for this analysis.

In thermal-stressed ovaries we observed upregulated heat-shock genes and

significantly differential expression of miRNAs, piRNAs and TEs. The most mobile TEs included DNA and LTR class TEs. In testes of thermal-stressed males, miRNAs exhibited significantly reduced expression, but TE transcript abundance and TE insertion mutation rates were increased across the genome. Kimura substitution level analysis showed expansion of the genomes in thermal stressed gonads by DNA and LTR TEs. TEs and piRNAs could be working antagonistically to invade and protect the germline. RNA-seq data showed some shared differentially expressed heat-shock genes, in the testes and ovarian tissues. Our results suggest that even short-term thermal changes have a marked effect on genome structure and increased TE activity, particularly in the male germline. These genomic changes in non-coding regions may contribute to inter- and trans-generational effects currently thought to be non-genetic.



How does adult age affect fertility following heat stress in male *Drosophila melanogaster*?

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MEETING ROOM 115, August 21, 2025, 10:30 AM - 12:30 PM

High temperatures can impair animal fertility, which could have major consequences for individual fitness and population persistence under a warming climate. For example, in some species of *Drosophila* fruit flies, heat stress causes permanent male infertility. Crucially, infertility can occur at temperatures up to 4°C lower than that at which death occurs, which could mean that we are underestimating the vulnerability of animal populations to future climate change. There is also evidence that different insect life stages can vary in their sensitivity of fertility to heat stress. We may therefore be underestimating vulnerability to warming even more if there are sensitive periods that we are missing. However, little is known about how this effect varies with age post-adult eclosion. Sexually immature adult males have lower levels of mature sperm stored in their seminal vesicles than mature adults and may therefore be more vulnerable to fertility loss following heat exposure. To investigate this, we exposed adult male

Drosophila melanogaster flies of three different age groups (1-6 hours, 1 day, or 7 days post-eclosion) to a range of heat stress temperatures (25, 36, 36.5 or 37°C) for four hours. The age treatments span the final stages of male reproductive maturation, which may be especially sensitive to heat stress. We measured the survival, fertility, mating behaviour and offspring production of these males to assess the impact of heat stress on survival and reproduction across these age groups. These results will help us to identify whether, and to what extent, this effect differs between adult age groups. If variation in heat stress vulnerability with age is found, this could have implications for how future climate change affects population structure, as the fitness of young and old individuals may be differentially impacted. Age may therefore need to be considered when assessing a species' thermal tolerance, as methods that only assess one age group could be under- or overestimating species' vulnerabilities to climate change.



Impact of thermal stress on colony foundation in ants

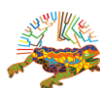
Roux A, Zimmermann A, Goubault M, Villalta I, **Libbrecht R**¹

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MEETING ROOM 115, August 20, 2025, 10:30 AM - 12:30 PM

Climate change not only leads to an increase in average temperatures but also to episodes of extreme heat. These thermal stresses impact the reproduction and survival of organisms, thereby influencing ecological balances and ecosystem functioning. These effects are exacerbated when thermal disturbances affect ecosystem engineers such as ants. The impacts of thermal stresses on ant populations have primarily been studied in mature colonies, which are relatively protected against thermal disturbances by the nest architecture and/or various behavioral responses. However, little is known about the effects of thermal stresses on founding queens that initiate new colonies. These founding queens are more exposed to heat stress, especially during mating and in the hours following the nuptial flight, usually occurring in summer.

To study the impact of thermal stress on ant colony foundation, we exposed founding queens of the black garden ant (*Lasius niger*) to a brief, acute heat stress just after the nuptial flight, before returning them to optimal conditions. We monitored queen survival and the number of eggs, larvae, pupae and workers produced. We found that the exposure of founding queens to the thermal stress impacted the success of colony foundation via increased queen mortality and reduced likelihood of producing workers, which stemmed from alterations of egg production and/or development. Our findings demonstrate impacts of climate change-associated thermal disturbances on colony foundation in ants, which, in turn, may alter ant population densities and more generally ecosystem functioning.



Influence of heatwaves on breeding success in the collared flycatcher (*Ficedula albicollis*)

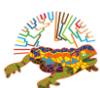
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MEETING ROOM 115, August 21, 2025, 10:30 AM - 12:30 PM

The influence of climate change on breeding success has drawn increasing interest from researchers. While numerous studies have investigated this topic, the results often vary across species, and many do not clearly identify which specific environmental factors most significantly affect the breeding process. However, a growing body of research highlights heatwaves as a critical factor affecting individual fitness. Even when temperatures remain within a species' thermal optimum, the accumulative effect of heatwave events can impact breeding outcomes and alter population dynamics. In this study, we investigated the influence of heatwaves on the breeding success of the collared flycatcher (*Ficedula albicollis*). Our

dataset comprised over 13,000 records of females, including data on the number of fledglings and recruited offspring between 1980 and 2017 in a Swedish population on the island of Gotland. For each female, we assessed whether she experienced heatwaves during the breeding season, and if so, how many such episodes occurred within a single season. The analyses also considered both the duration and intensity of these heatwaves. Remarkably, between 20% and 30% of females encountered heatwave events during either the incubation or fledging stages. We tested and discuss the hypothesis that heatwaves negatively impact breeding success of the collared flycatcher.



Life-stage- and sex-specific fitness dynamics of thermal stress intensity and duration in *Drosophila prolongata*

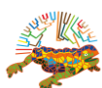
Meena A¹, Cortés Mediavilla M¹, Eggs B¹, Raina G¹, Sbilordo S¹, Meena S¹, Jiang J¹, Nardo A¹, Ratz T¹, Lüpold S¹

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MEETING ROOM 115, August 20, 2025, 10:30 AM - 12:30 PM

Global climate change is intensifying the frequency and severity of extreme heat events, posing significant risks to biodiversity and ecosystem stability. While many studies examine species survival in response to these events, long-term population persistence also critically depends on reproductive success, a trait often more susceptible to thermal stress. Sublethal temperatures can compromise gamete and gonad function, and recent studies suggest that reproductive sensitivity varies across life stages and between sexes. However, most investigations are limited in scope, focusing on single developmental stages, sexes, or exposure durations. To address these limitations, we investigated the impacts of short-term heat stress on survival and reproductive fitness in *Drosophila prolongata*. Specifically, we examined: (i) how heat stress affects survival and reproduction across developmental stages;

(ii) whether sex-specific differences exist in thermal sensitivity; (iii) how the intensity and duration of thermal exposure modulate these effects; and (iv) the underlying mechanisms and strategies mediating fitness consequences. Our findings reveal that survival is most vulnerable during early developmental stages, whereas reproductive traits are most affected in later stages. Both sexes are impacted by heat stress, but males display a more pronounced decline in fertility, associated with damage to reproductive tissue. These findings underscore the importance of integrating life stage- and sex-specific responses, as well as mechanistic insights, into climate resilience assessments for biodiversity conservation. This study is particularly pertinent for insects, given their rapid global decline and direct dependence on environmental temperature.



Recombination Plasticity in Response to Temperature Variation in Reptiles

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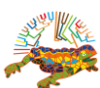
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MEETING ROOM 115, August 21, 2025, 10:30 AM - 12:30 PM

Reproductive processes are particularly vulnerable to environmental changes, especially in ectothermic species whose physiology is directly influenced by ambient temperature. While it is well-established that organisms activate common transcriptional pathways in response to temperature variations, the impact of temperature on recombination, a key source of genetic variability, remains largely unexplored. Previous studies in model species have shown that the frequency of recombination during meiotic prophase I can be influenced by extreme temperatures. Yet, it remains unclear whether this effect is also conserved in non-model vertebrates, especially in natural populations. In this study, we investigated the effect of temperature on meiotic recombination in the Guibé's ground gecko (*Paroedura guibeae*), an ectotherm species. We analyzed the formation of

double strand breaks (DSBs) and crossovers (COs) by immunolocalizing the meiotic proteins involved in these processes. Additionally, we determined the frequency and chromosomal location of COs and assessed the levels of CO interference. Our findings reveal the presence of hyper-COs spermatocytes in individuals exposed to both high and low temperatures. Notably, this marked increase in COs was accompanied by shorter chromosome axis lengths and higher levels of DSBs in later stages of meiosis. These findings suggest that environmental temperature shifts can disrupt meiotic dynamics, with potentially affecting fertility and species adaptation. Overall, this study highlights a mechanistic link between environmental stressors and reproductive outcomes in ectothermic species.



Reproductive success under hypoxia: unveiling the adaptive potential of a freshwater fish

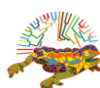
Crespel A¹, Valvandrin A¹, Bouqueau M¹, Grassin T¹, Bermejo Albacete L¹, Anttila K¹

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MEETING ROOM 115, August 20, 2025, 10:30 AM - 12:30 PM

While hypoxia (i.e. condition of low dissolved oxygen level in water) events occur naturally, global change and human activities have exacerbated their strength and temporal fluctuations. As populations may not be able to escape the hypoxic conditions, especially in freshwater ponds, it is crucial to understand how and through which mechanisms organisms can adapt to these fluctuating environments. When directly exposed, individuals may be able to adjust their phenotypes through plasticity. However, continued response over generations may require adaptation and evolution. As a good reproductive success is essential for the persistence of populations across generations, directly shaping their adaptive potential, a decline of its quality is likely to have severe consequences on the ability of populations to cope with environmental changes. However, so far, little is known on how the plasticity and/or adaptive potential of reproductive success itself might help species to survive under hypoxia. Here we used two populations of wild three-spined sticklebacks

(*Gasterosteus aculeatus*) collected from streams already experiencing and potentially adapted to strong hypoxia events or not. We exposed these two populations to experimental fluctuating hypoxic conditions that would happen in the wild (oxygen level about 30% air saturation during the night and 100% during the day) and evaluated their breeding success (egg quality, hatching rate) and larvae fitness (survival, growth, metabolism). We showed an overall reduction of the reproductive success in the population previously adapted to hypoxia events, and this reduction was even more pronounced when the offspring were raised under normoxia. Such reduction could have strong evolutionary consequences on the persistence of the species. Our findings highlight that even ongoing adaptation to hypoxia might lead to challenges in maintaining reproductive success, rising concerns that the long-term effect on reproductive capacity could ultimately threaten the populations persistence to rapidly changing environments.



The effect of of intrasexual and intersexual selection on survival and reproduction under a heatwave.

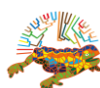
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MEETING ROOM 115, August 20, 2025, 10:30 AM - 12:30 PM

Heatwaves are set to become more common due to climate change, and the potential of heatwaves to damage a species' populations is becoming more apparent. One way heatwaves can affect reproduction is by changing the propensity of pre-copulatory mating behaviours, which can impact individuals' fitness by impacting their survival and reproductive success. However little work has been done to investigate how different levels of intrasexual and intersexual selection in pre-copulatory behaviours, such as male-male competition or female-mate choice modulate fitness responses to heatwave. Here we investigate how differing levels of male competition and female choice impact survival and reproductive success, when a heatwave event occurs using the burying beetle *Nicrophorus vespilloides* as a model species. We had

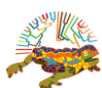
three treatments for pre-copulatory conditions: monogamy (one male, one female, no male-male competition), polyandry with male competition (one female two males, where males and females could freely interact), and polyandry without male competition (one female two males, where males could not interact with each other or the female freely) and subjected the beetles to a heatwave event (3 days at 25°C) at the time of mating. We found that the likelihood of having a brood decreased under a heatwave when males could not freely compete or harass the female, suggesting that the thermal stress decreased female engagement in mating, thereby reducing reproductive success. Our results indicate that differing levels of intrasexual and intersexual selection can impact heatwave responses and fitness.





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S45 - The evolution of microbial pangenomes



Bacteriocin competition: ecological and evolutionary dynamics in colonization

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POSTER SESSION 3 - **P03.218**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

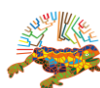
Bacteriocins are narrow spectrum antimicrobial compounds created by bacteria to kill closely-related taxa competing in the same niche, with between-strain differences in bacteriocin production, immunity, and regulation contributing to intra-strain competition for host colonization. Bacterial genomes encode for multiple bacteriocin systems which exhibit extreme diversity both within and between themselves—meaning ecological interactions are determined by immunity and susceptibility across many loci—but it remains unclear what patterns of diversity emerge when competition is driven by difference in multiple traits. Here, we link ecological modeling with genomic analysis to explain the diversity across and within bacteriocin loci, as well as investigate the role of bacteriocins in the epidemiological dynamics of *Streptococcus pneumoniae* colonization in humans.

We demonstrate an ecological model of bacteriocin-mediated competition which builds on previous work by explicitly considering the impact on strain coexistence at the host population level when colonizing

strains possess multiple independent bacteriocin systems. The model predicts strain coexistence as a function of epidemiological and ecological parameters, allowing us to estimate stable phenotype distributions that maintain microbe diversity across the host population.

Additionally, we share results from screening publicly available *S. pneumoniae* genomes for the presence of known bacteriocin genes. This allows us to characterize the diversity of *S. pneumoniae* populations as they exist in situ, as well as reconstruct the ancestral states of our strains to conduct further phylogenetic analysis. Doing so reveals patterns of toxin and immunity genes persisting at intermediate frequencies across bacteriocin loci, supporting results from our model that bacteriocin-mediated competition promotes strain diversity.

Future work will further develop our analysis of the evolutionary epidemiology of bacteriocins using comparative phylogenetics, with the goal of using our theoretical insights to explain patterns in the sequencing data.



Phylogeny, evolution and virulence of *Coxiella burnetii* the agent of the Q fever

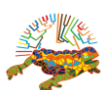
Tricou T¹, Jourdain E¹, Thézé J¹, Bailly X¹

¹EpiA Inrae

POSTER SESSION 3 - **P03.219**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Coxiella burnetii, the agent causing widespread zoonosis Q fever, is a strictly intracellular bacterium. In Humans, infections can range from asymptomatic to flu-like to more severe and rarely lethal. Domestic ruminants, such as goats, sheep, and cattle, are considered to be the main reservoirs. In animals, Q fever is often asymptomatic but may lead to abortions and stillbirths, which may translate into significant economic losses. Although *C. burnetii* has been studied for decades, its genetic diversity remains understudied, and our understanding of the potential links between genotypes and virulence traits is limited. Currently, only a few full genome sequences of *C. burnetii* are available, and most sequencing data are obtained from old laboratory isolates. In addition, axenic cultivation from some genotypes has proven difficult and inefficient.

In this work we use more than 250 *C. burnetii* strains including 60 novel genomes from the Q-NET-ASSES European ICRAD-project, to investigate *C. burnetii* evolution, genetic diversity, metabolism specificity in relation to host and geographical origin. We reconstruct a core-genome phylogeny and identify 9 main clusters. The two most distant clusters accounted for most European small ruminant strains. They are also the most genetically diverse when compared to two cattle specific clusters or the Nine-Mile cluster. Interestingly, strains from these two clusters were also the most recalcitrant to culturing in axenic media. Pan-genome analysis and metabolic annotation have identified multiple cluster-specific gene contents and metabolic specializations that might be linked to host specificity, virulence, or the difficulty in cultivating certain strains in axenic media.



Recombination enforces diversity of secreted proteins in a symbiont of honeybees

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POSTER SESSION 3 - **P03.217**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Secreted proteins from lactic acid bacteria have gained attention because of their applications in the food industry. In *A. kunkeei*, a bacterial symbiont of honeybees, some of the most abundant secreted proteins are glycosyl hydrolases, which participate in carbohydrate metabolism. *A. kunkeei* contains a region of the genome with accessory genes that encode glycosyl hydrolases from families 70 and 32 (GH70 and GH32, respectively). Enzymes of the GH70 family metabolize sucrose by synthesizing glucose polymers such as dextran while enzymes in the GH32 family synthesize fructose polymers. The region of the genome with glycosyl hydrolase genes shows divergent gene combinations depending on the strain, while the neighboring regions are conserved. Most research on GH70 and GH32 has focused on their structure and function, with few investigations of their unique patterns of evolution.

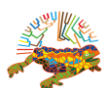
In this study, we aim to uncover the evolutionary mechanisms that shape the genomic region with glycosyl hydrolases in *A. kunkeei*. For this, we studied the presence and absence patterns of genes in the region, characterised sequence domains and motifs, built protein domain phylogenies, calculated rates of evolution and ran recombination analyses. We also used ClonalFrameML to check for the overall effects of mutation and recombination in the *A. kunkeei* phylogeny.

We found a great diversity of glycosyl hydrolases, whose presence and absence patterns were inconsistent with the strain phylogeny. Most glycosyl hydrolase genes contained a signal peptide, a glycosyl hydrolase domain and glucan-binding repeats.

The GH70 genes in the region could be classified as glucansucrases (GS) or branching sucrases (BRS) by comparing them to sequences of known function, but we found two additional GH70 genes which have not been functionally characterized so far. We found remnants of GS and BRS in strains which had partially lost the genes. The GH32 genes in the dynamic region were divided into three subtypes based on their phylogeny.

We found patterns of evolution that differed to those of core genes, with a prominent role of recombination in the glycosyl hydrolase region and in the *A. kunkeei* genome at large. In particular, the rates of gene evolution of GH70 and GH32 were generally faster than the average rates of core genes. The glycosyl hydrolase region underwent putative recombination events over long tracts and shorter recombination events involving single domains or gene segments. For GH70-protein encoding genes, we found that certain GS contain sequence repeats that could enable the insertion of a BRS domain, which results in GS-BRS genes. Overall, we found that recombination has an effect 2-5 times larger than mutation in the *A. kunkeei* genome.

We conclude that glycosyl hydrolase genes show great diversity and evolve differently than core genes. Glycosyl hydrolase genes seem to be frequently lost, but may re-insert into the genome through recombination, either as single domains or as part of a large segment. We hypothesize that the constant gene losses and re-gains create diverse gene combinations which might be important for the bacterial colonization of the sugar-rich environments associated to honeybees.



Emergent epistasis mediates the role of negative frequency-dependent selection in bacterial strain structure

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MEETING ROOM 129+130, August 22, 2025, 1:30 PM - 3:00 PM

The pangenome of many pathogenic and commensal bacterial species is structured: distinct strains persist over time exhibiting stable associations between genetic or phenotypic traits, a phenomenon referred to as strain structure. This structure is surprising, particularly in highly recombinogenic species like *Streptococcus pneumoniae*, because recombination typically breaks down linkage disequilibrium (LD), i.e. the non-random association of alleles at different loci.

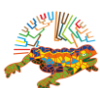
Allelic diversity is a necessary condition of strain structure. Recent work has shown that multi-locus negative frequency-dependent selection (NFDS) acts to maintain diversity across bacterial genomes. Here, using modelling and genomic analysis, we show that multi-locus NFDS also shapes bacterial strain structure through emergent epistatic effects.

We first develop mathematical models of two NFDS mechanisms: metabolic niche differentiation and competition-colonisation trade-offs. We show that epistasis emerges readily in these models. Notably, both models generate frequency-dependent epistasis. Unlike classical epistasis, this acts to either reinforce or abolish existing strain structure, depending on how NFDS is assumed to combine across loci. The direction of LD maintained through

frequency-dependent epistasis is contingent on the evolutionary history of the population. Importantly, both models are only neutral with respect to strain structure (i.e. neither strengthening or reinforcing existing LD) under very specific assumptions, which there is no a priori reason to expect to hold.

We then use a dataset of over 3000 *S. pneumoniae* genomes to test our model predictions, and make observations consistent with emerging epistatic effects. Specifically, we compare patterns of association between intermediate frequency genes across three different populations. We find that genes in strong LD in one population are more likely than expected to be in strong LD of the same sign in other populations (consistent with all forms of epistasis), but also strong LD of the opposite sign (consistent with frequency-dependent epistasis).

Our results extend and generalise previous work on the role of antigen-specific acquired immunity (a diversity-maintaining mechanism) on strain structure. Our work contributes to a better understanding of the evolutionary processes shaping the structure of bacterial pangenomes, which is central to predictive modelling of multi-strain pathogens.



Solving the pangenome paradox: detecting accessory genes under balancing selection in *Klebsiella pneumoniae*

Conradsen C^{1,2}, Vos M^{1,2}, Eyre-Walker A³, Feil E⁴, Bayliss S⁵

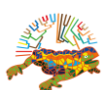
¹European Centre for Environment and Human Health, University of Exeter Medical School, Environment and Sustainability Institute, ²Environment and Sustainability Institute, University of Exeter, ³School of Life Sciences, University of Sussex, ⁴Department of Life Sciences, University of Bath, ⁵Bristol Veterinary School, University of Bristol

MEETING ROOM 129+130, August 22, 2025, 1:30 PM - 3:00 PM

The pangenome of bacterial species is characterised by a diverse accessory component, yet its contribution to bacterial evolution remains paradoxical. While some accessory genes are clearly adaptive (e.g. antibiotic resistance genes), others may be functionally disruptive or confer no discernible advantage. Persistent accessory genes are expected to evolve under balancing selection, potentially driven by fluctuating selection across space or time. However, testing for selection that maintains genes at intermediate frequencies remains challenging. The inconsistent presence of accessory genes across bacterial strains complicates efforts to apply conventional population genetic models, which largely focus on testing departures from neutrality for shared genetic variation.

Here, we present a population genetic test that uses the frequency and inferred age of

accessory genes, with age estimated from sequence divergence between genomes sharing each gene, to detect signatures of balancing selection in a high-resolution *Klebsiella pneumoniae* pangenome dataset. Building on the expectation that genes under balancing selection maintain elevated nucleotide diversity, occur at higher intermediate frequencies within populations, and persist over longer timescales than those evolving neutrally or under purifying selection, we model a null distribution to identify empirical outliers indicative of non-neutral evolutionary dynamics. We then incrementally refine our models by incorporating demographic effects and drawing genes from a distribution of fitness effects. This work helps reconcile theoretical models with empirical data and introduces a new approach for investigating pangenome evolution.





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**S46 - The future meets the beginning:
Synthetic biology, evolution, and the
origin of life**



Applying Statistical Models to the Family Resemblance Definition of ‘Life’

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POSTER SESSION 1 - **P01.342**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The question of how to define life has been unresolved in the philosophy of biology for many years, but developing a definition of life that is useful in both technical and everyday contexts has become more urgent as researchers around the world attempt to create fully synthetic cells in the laboratory, develop more and more intelligent and autonomous robots, and search for signatures of life elsewhere in the galaxy. Developments in these areas may end up overturning our current ideas about the distinction between life and non-life. It is therefore important to consider whether it is possible to develop a definition of life that encompasses currently known lifeforms, while at the same time having the potential to be applied to as-yet unknown lifeforms. The typical approach to defining life is to use a so called *de re* definition, that is, to make a list of necessary properties that together makes up a sufficient set of criteria for being alive. This is not the only possible way of constructing a definition, however. An alternative approach would be Wittgenstein’s family resemblance definition, according to which a concept is not defined by necessary and sufficient properties but by a cluster of properties that are associated with the concept. If we use this concept to define Life’ it tells us that something is alive if it has a number of properties that are associated with being

alive though it does not have to have all these properties and it does not have to have exactly the same set of properties as any other living entity. In this paper we present an extension of the family resemblance model by combining it with methods for statistical modelling in the form of cluster analysis to test different properties regarding their ability to predict whether a certain entity is commonly considered to be alive, and also, given a certain set of criteria, test if a certain borderline case can qualify as being alive or not. In order to compile a list of properties to test we scanned the literature for properties that have been suggested as being strongly associated with life. From these we compiled a list of proposed criteria for defining life, as well as suggested “borderline” or “problematic” test cases (e.g. viruses) when defining living versus nonliving. In addition to the test cases we selected a representative list of taxa to which we could apply the criteria we compiled. We found that although living and non-living entities can be grouped according to overall similarity, it is difficult to find a single set of criteria which is sufficient for defining known forms of life while at the same time being inclusive enough to be useful in identifying or characterizing novel forms of life. We hope that the family resemblance approach will prove to be a fruitful alternative to traditional approaches to defining life.



Disentangling the evolutionary impacts of relatedness and facultative/obligate life cycles during the transition to multicellularity

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POSTER SESSION 1 - **P01.340**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Complex multicellularity evolved in five lineages, and in each case, these organisms developed clonally and are obligately multicellular. While prior work has shown that clonal development plays a critical role in the evolution of complex multicellularity, none has disentangled this from the impact of obligate vs facultative multicellular life cycles. Theory predicts that selection acting on single cells may impede group-level adaptation, but due to a lack of model systems, this has never been tested. We circumvent this constraint by engineering a strain of *Saccharomyces cerevisiae* to grow as single cells or clonally-multicellular groups ‘snowflakes’ upon induction with β -estradiol. We evolved 20 replicate populations of one ancestral genotype with four different life cycles: always unicellular, always multicellular, or alternating between uni and multicellularity (either spending 50%

or 75% of their time in a multicellular state). Only the obligately multicellular treatment evolved ‘entrenching’ traits: those which increase group-level fitness at a cost to cell-level fitness. For example, obligately multicellular snowflake yeast convergently evolved to be tetraploid, increasing group size and fitness but reducing cellular growth rates. We show that while tetraploidy should be adaptive in the facultatively multicellular treatments, it never evolved, because selection in the unicellular stage is far more effective than the multicellular stage (~100-fold higher N_e). Our results demonstrate that an obligately multicellular life cycle plays a key role in the transition to multicellularity independent from its effect on genetic relatedness, by avoiding disproportionately powerful cell-level counterselection against novel multicellular traits.



The Race Between Entropy and Maximum Entropy: Balancing Innovation and Survival

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¹Charles University

*POSTER SESSION 1 - **P01.341**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM*

Living systems persist by maintaining a structured order against increasing local entropy. Building upon Brooks and Wiley's (1986) view of evolution as an entropic process where the local maximum entropy (S_{max}) of a system increases over time, I propose that life can be understood as a dynamic race between actual local entropy (S) and expanding local maximum entropy (S_{max}). Death — both at the individual and species levels — occurs when organisms fail to maintain sufficient internal constraints to

keep pace with the growing potential for disorder. Furthermore, I extend this view to species lines and evolutionary trajectories, arguing that increasing the space of available configurations offers potential for complexity but also elevates the risk of structural failure. This view unifies biological mortality with thermodynamic inevitability, and refines earlier theories by explicitly formalizing the relationship between constraint maintenance and entropy expansion.



Emergence, Evolution and Functional Properties of Random and de novo Proteins

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MEETING ROOM 131, August 18, 2025, 2:00 PM - 3:30 PM

Background: Sequence-space of proteins is vast, yet nature has managed to generate many functional proteins that cluster into evolutionary families. Despite the enormous size of sequence-space, it remains unclear how proteins initially arise and whether they can evolve from random sequences within this vast and largely unexplored region, reminiscent of the dawn of life. It is also uncertain whether and how proteins from one evolutionary cluster can transform into those from another, as the "fitness valley" that needs to be crossed appears deep and non-permissive, regardless of the mutational processes (point mutations, recombination).

Rational approaches to identify these "islands" of functional, marginally stable, well-conserved, and structured "canonical" proteins from random sequences, and to design transitional pathways connecting them, have largely been unsuccessful. Well-understood mechanisms of protein evolution often follow a "new-from-old" principle. These processes include gene duplication followed by sequence and functional divergence (neo- or sub-functionalisation), and modular rearrangements of domains—evolutionarily conserved structural and functional building blocks. Over the past 15 years, studies across various organisms suggest that novel "de-novo" proteins—previously unseen and unevolved—may emerge from non-coding regions through a series of smaller mutations that enable transcription, translation, and functionalisation.

Understanding how such evolutionary "unapproved" proteins, which are typically disordered and of unknown function, manage to evade purging and eventually become essential could provide insights into the fundamental capacities and limitations of proteins and shed light on principles underlying all forms of life, from ancient to extant.

Methods: We studied thousands of random sequences reflecting the natural distribution of extant and ancient amino acid distributions. These were compared to putative de-novo proteins derived from a broad range of data, including populations and closely related species of flies that

were reared under controlled conditions. Their genomes and transcripts were sequenced, annotated, and further analysed.

We employed large libraries and performed Tat-export assays and Lon-protease assays to assess folding and solubility, and FRET assay combined with FACS-sorting and NGS-sequencing to determine foldability, compactness, and disorder content.

Results: De-novo transcripts are primarily intronic, often intergenic, sometimes anti-strand, and mostly short and intron-less. The encoded proteins are mostly disordered and, in comparison to canonical proteins, not compact. However, like random proteins, many display secondary structural elements, interact with chaperones, and occasionally exhibit beneficial functions. Relative to random proteins, de-novo proteins are more prone to degradation, slightly more soluble, and more disordered. Their solubility can be enhanced by chaperones, which likely reduce their toxicity compared to random proteins. Iterative FACS sorting of FRET-positive de-novo proteins reveals that a small fraction is highly compact.

A comparison with models that calculate the probabilities for the random emergence of DNA motifs promoting transcription and translation shows that the early stages of de-novo gene evolution can be effectively explained by a neutral model.

Conclusions: Our results suggest that many de-novo and random proteins share structural and folding characteristics that make them suitable for cellular interactions, which may expose them to potentially beneficial mutations.

Since their emergence and early evolution can be effectively explained by stochastic processes, we conclude that the observed relative overrepresentation of longer proteins among older de-novo proteins can be attributed to the generally higher folding probability of longer proteins, despite their relative rarity.

In other words, "only the long survive," and they were "born this way."



Order of amino acid recruitment into the genetic code resolved by last universal common ancestor's protein domains

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MEETING ROOM 131, August 18, 2025, 2:00 PM - 3:30 PM

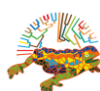
The order in which the amino acids were added to the genetic code was previously inferred from consensus among forty metrics. Many of these metrics reflect the physical and chemical environmental factors present on ancient Earth, such as abiotic abundance of amino acids. However, estimates of abiotic abundance do not necessarily reflect biological need and in some cases, may be even flawed. For instance, the sulfur-containing amino acids, methionine and cysteine, were assumed to be late additions to the code due to their absence in the products of the Urey-Miller experiment which lacked sulfur altogether. Our research takes a different approach; asking which amino acids were more frequently present in sequences born during the construction of the code instead of relying on what chemistry deems feasible.

The genetic code is assumed to be completed by the time of the last universal common ancestor (LUCA). Previous studies focused on identifying orthologous protein families that date back to LUCA to infer early life metabolism. Our research is the first to date protein domains — the basic evolutionary units of proteins that evolve and fold independently. Proteins are often made of multiple domains, each of which might have originated at a different point in time. We classified the relative age of protein domains based on their presence in LUCA, the last bacterial common ancestor, the last archaeal common ancestor and the last common ancestors of prokaryotic supergroups.

We inferred the order of recruitment of amino acids into the genetic code from the ancestral amino acid usages of protein domains dating back to LUCA relative to more recent, yet still-ancient, post-LUCA domains that date back to the last bacterial and archaeal common ancestors. We find that smaller amino acid size predicts ancient amino acid enrichment better than the previous consensus metric does. Our new phylogenetically inferred order also places metal-binding (histidine and cysteine) and sulfur-containing (cysteine and methionine) earlier than previously thought. The latter finding highlights the importance of metal-dependent catalysis and sulfur metabolism to ancient life, suggesting a metal- and sulfur-rich environment was likely conducive to the origins of life on earth.

More ancient pre-LUCA sequences, which had already diversified into multiple copies prior to LUCA, exhibit significantly higher frequencies of ring-containing amino acids than LUCA. Some pre-LUCA sequences could have emerged in now-extinct non-LUCA lineages and were then horizontally transferred to LUCA. It is then possible that some pre-LUCA sequences predate the current code, and their distinct amino acid usage reflects earlier, alternative codes.

Understanding the origin and evolution of the genetic code sheds light onto the origins of life on Earth, informs the field of synthetic biology, and guides our search for life elsewhere in the universe.





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S47 - The interplay between genetic architecture and the evolution of biodiversity



A moving avian hybrid zone provides insights into how the genomic architecture of traits under natural and sexual selection shapes speciation

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POSTER SESSION 2 - P02.333, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Hybrid zones are areas where genetically differentiated groups of individuals meet, mate, and produce offspring of mixed ancestry. These areas offer direct insights into traits involved in reproductive isolation and their genetic architecture. Sexual selection can promote speciation in its early stages, and its effects are amplified when combined with natural selection, facilitating reproductive isolation and speciation. A better understanding of the genomic basis of traits underlying reproductive isolation enables us to unravel how populations and species have evolved and how genomic differentiation may respond to changing environments. However, the genetic architecture of reproductive isolation can be obscured by other evolutionary processes unrelated to speciation, such as recombination rate variation and gene density. Systems marked by strong sexual selection and extensive gene flow can provide a clearer picture of number, location and genetic architecture of barrier loci involved in early stages of speciation. Here, we study the moving hybrid zone between two sister species, black-headed and red-headed buntings, which are notable for their significant differences in male plumage, likely driven by sexual selection.

To test if species boundaries are maintained by natural selection, we modeled their suitable ecological habitats in the present and during the Last Glacial Maximum. We also performed whole-genome resequencing of 38 museum and fresh specimens sampled from across the entire distribution range, including the hybrid zone, to reconstruct the demographic history of species formation. We conducted genomic scans to assess the interplay among demography, selection, and genetic architecture. We used coalescent analyses to investigate how genetic architecture has shaped the dynamics of genomic differentiation in response to climate change.

Our ecological modelling demonstrates that the two species have distinct ecological

preferences, suggesting that natural selection and adaptation to specific ecological niches may contribute to speciation. Demographic analyses based on genomic data indicate a very recent divergence time, with strong asymmetric gene flow from the native black-headed to the invading red-headed buntings. Our genomic results confirm several generations of backcrosses in the hybrid zone, revealing that reproductive isolation is still incomplete. Consistently, our genomic scans show that only 1.5% of the genome exhibits elevated F_{ST} , contrasting with generally low genome-wide F_{ST} ($= 0.06$). These outlier regions are scattered across the genome. A positive correlation between F_{ST} and D_{XY} in some of these outliers highlights them as candidates for reproductive barriers under selection rather than products of long-term linked selection unrelated to speciation. These regions also show distinct polymorphic and fixed allele patterns, indicating localized selection pressures possibly linked to plumage coloration or ecological adaptation. In contrast to neutral regions homogenized by extensive gene flow and showing short coalescence times, these outlier regions form distinct clades with deep coalescence.

Our study shows that using a moving hybrid zone provides unique insights into the genomic architecture of speciation because of extensive homogenizing gene flow throughout most of the genome. It allows us to unravel the dynamic trajectory of genomic differentiation and predict how different parts of the genome may respond to changing environments. We identified a few candidate loci with large effects, spread across the genome, with a simple architecture related to reproductive isolation. Despite extensive gene flow that has homogenized most of the genome, natural and sexual selection maintain differentiation at these key loci, even in the face of significant range shifts.



A single theory for the evolution of sex chromosomes and the two rules of speciation

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*POSTER SESSION 2 - **P02.313**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM*

Sex chromosomes are involved in three major empirical patterns: Y (or W) chromosomes are often non-recombining and degenerate; heterogametic offspring (XY or ZW) from interspecific crosses are more often sterile or inviable than homogametic offspring (Haldane's rule); the X (or Z) has a disproportionately large effect on reproductive isolation between species compared to autosomes (the large X effect). Each observation has received its own tailored explanation involving multiple

genetic and evolutionary causes. Here, we show that these empirical patterns all emerge from a single theory for sex chromosome evolution incorporating the co-evolution of cis and trans-acting regulators of gene expression, and leading to systematic misexpression of dosage-compensated genes in heterogametic F1 hybrids, for both young and old sex chromosomes.



Adapting complex phenotypes despite pleiotropy: the importance of regulatory networks structure

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POSTER SESSION 2 - **P02.381**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Complex traits are determined by many loci—mostly regulatory elements—that, through combinatorial interactions, can affect multiple traits. Such high levels of epistasis and pleiotropy have been proposed in the omnigenic model and may explain why such a large part of complex trait heritability is usually missed by genome-wide association studies. This genetic architecture could also limit the possibility for a specific complex trait to be involved in adaptation processes without creating potentially deleterious changes to another one. However, the literature is full of examples of complex traits involved in the adaptation to the environment in many different species. In the last few years, systems biology and in particular gene regulatory networks offered a new perspective on the molecular bases of complex traits, showing that highly structured networks regulate the expression of complex traits. However, the link between this regulatory-centric representation of complex traits' molecular bases, their genetic architecture and their evolution is still unclear.

Materials and Methods: To tackle this question, we used the GTEX dataset to build 9 tissue-specific regulatory networks built from expression Quantitative Trait Loci (eQTL) results. We then systematically analyzed the distribution of SNP heritability for eleven traits across those networks. Finally, we studied the distribution of

molecular signatures of past and ongoing selection events in these networks.

Results: We found that heritability for a given trait is clustered in a small number of tissue-specific, functionally relevant SNP-gene modules. The greatest heritability occurred in local “hubs”. These hubs, that are the cornerstone of the network's modules, correspond to tissue-specifically activated regulatory elements that affect the expression of many genes involved in a particular biological function without impacting other functions. We also found that these local “hubs” are enriched for molecular signatures compatible with past and ongoing polygenic directional selection events.

Conclusions: Together, these results provide a conceptual framework for understanding the architecture of complex traits and their evolution. The highly structured topology of eQTL networks and the presence of local “hubs” can both amplify the genotype-phenotype connection by allowing one genetic variation to affect the expression of genes in a whole pathway, and buffer the deleterious effect of this same variation on other pathways. This effect is reinforced by the tissue-specific regulation of gene expression. Despite the pervasive pleiotropy, network structure thus opens a path to understand how complex traits can contribute to the adaptation to new environment.



An empirical fitness landscape of gene regulation in E.coli

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POSTER SESSION 2 - P02.318, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Regulation of gene expression is fundamental to all cellular life. Gene expression carries a cost in all environments, because it consumes energy and materials for mRNA and protein production. In microbes, this cost can reduce growth rate and fitness. Any benefit a gene provides needs to be larger than this cost. Both cost and benefit may depend on a gene's expression level and may vary with the environment. To attain high fitness in any one environment, an organism must thus be able to regulate gene expression according to the environment. This regulation is primarily exerted by transcription factors (TFs). These proteins bind specific DNA sequences called transcription factor binding sites (TFBSs). In this way, TFs either activate or repress the transcription of target genes. The fitness of a TFBS can depend on its ability to balance repression and activation across environments.

In this study, we aim to understand how evolution might maintain a balance between repression and activation (de-repression) of gene expression across environments. To do so, we examine a regulatory fitness landscape in two distinct environments: one where a gene needs to be repressed because it conveys no benefit, and another where this same gene needs to be activated (de-repressed). While several studies have explored fitness landscapes in multiple environments—particularly in the context of protein evolution, these landscapes are typically small in scale and focus on amino acid changes that affect protein function or stability. Such studies have provided valuable insights into how proteins adapt to shifting selection pressures. In contrast, much less is known about how regulatory DNA evolves to optimize gene expression across environments, leaving a crucial question unanswered: How does

regulatory DNA evolve to optimize gene regulation and contribute to an organism's fitness across different environments?

We chose the catabolic lactose (lac) operon of *Escherichia coli* as a model system for studying the fitness landscape of prokaryotic gene regulation. To metabolize lactose *Escherichia coli* cells must express the genes of the lac operon. In the absence of lactose, this operon is repressed by the transcriptional regulator LacI, which binds to lac operator DNA and prevents operon transcription. Because few naturally occurring binding sites of this operator are known, we know little about how operator variation may affect fitness. In addition, we do not know whether adaptive evolution can easily create strong operators from weak operators or de novo from non-regulatory DNA. To find out, we used CRISPR-Cas-assisted genome editing, bulk competition, and high-throughput sequencing to measure the fitness of more than 0.2 million lac operator variants. We mapped the lac operator fitness landscape not only in lactose but also in glycerol, where lac operon expression is costly and not beneficial. Both landscapes are highly rugged and contain thousands of fitness peaks, which allow only 2 percent of evolving populations to reach one of the top 100 peaks. From any one non-peak operator genotype, more than one top 100 fitness peaks are accessible by fitness-increasing mutational paths in both landscapes. Multiple lac operators with greater fitness than the wild-type exist in both environments, but in general *E.coli* is better adapted to lactose than to glycerol. Our work illustrates that landscape ruggedness caused by epistasis can represent an important obstacle to adaptive evolution, and that fitness landscape topography can be materially influenced by the environment.



Ancestral Hybridisation Events in Snapdragons

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POSTER SESSION 2 - P02.359, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Species radiations are important for generating biological diversity, however the drivers behind these radiations are not always well understood. One potential important driver of rapid speciation is hybridisation, which can facilitate the transfer of variants that selection can then act on. Within the rapidly radiating plant genus *Antirrhinum* (Snapdragons), hybridisation has been observed across the genus in the wild, but it is unknown if ancestral hybridisation events have helped shape the evolution of the genus. This study aims to determine whether there is evidence for shared ancestral hybridisation events across species within the *Antirrhinum* genus. Using whole genome sequence data from 18 *Antirrhinum* species, D-statistic (ABBA-BABA) analyses were carried out to identify

hybridisation events involving multiple species, suggesting the involvement of their common ancestor. To investigate these events further, D-statistics, along with diversity and divergence metrics, were calculated for sliding windows along the genome to locate genomic regions with signal of introgression and to determine if these regions are shared across all species involved in potential ancestral hybridisation events. Multiple ancestral hybridisation events were identified, with genomic regions with signal of introgression shared across involved species. This indicates that selection may have preserved these genomic regions and suggests that these ancestral hybridisation events have played an important role in the evolution of the *Antirrhinum* genus.



Avian structural coloration tuning explained by changes in feather keratinization

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*POSTER SESSION 2 - **P02.301**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM*

Coloration is a fascinating aspect of avian biology. The vast diversity of hues and patterns observed among birds is shaped by natural and sexual selection as a result of its high ecological impact, in particular for sexual attractiveness, communication and camouflage. Bird colors can emerge from the deposition of pigments throughout feather development and/or from the interaction of light with nanostructures within the feathers. Among the latter are colors such as opaque blues and violets, known as non-iridescent structural colors. This mechanism of color production occurs within feather barbs and relies on two principles: absorption of light by a proximal layer of melanin; and refraction of short wavelengths by interaction with a “spongy-like” matrix formed by keratin and air spaces. While this is well established, the

genetic bases of non-iridescent structural coloration in birds and how it is formed during feather development have remained poorly understood. Here, we combine photonics and microscopy to characterize an artificially selected mutation resulting in different shades of blue in lovebirds, which is representative of the natural variation in non-iridescent structural coloration observed among birds. Finally, genetic mapping along with gene expression analyses (including in situ hybridization, bulk and single-cell transcriptomics) uncovered a novel molecular mechanism based on protein cross-linking that can explain both the formation of opaque blue feathers, as well as the fine tuning of its hue, bringing a relevant advance in the field.



Changes in the cell migratory environment as a driver of irregular pigment cell migration in the blotch color polymorphism of African cichlid fishes

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POSTER SESSION 2 - **P02.317**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Color pattern formation is a fundamental part of development for many animals and plays a vital role in camouflage, mating, and species recognition. The study of pigmentation patterns offers valuable insights into molecular and cellular mechanisms that drive such complex biological traits. *Haplochromis chilotes*, a species of cichlid fish, exhibit a naturally occurring mutant phenotype in the form of a blotch that presents a model for studying pigmentation patterning and maintenance. This blotched phenotype, which is persistent in wild populations, is composed of melanophores—dark pigment cells—whose behavior differs across the various regions of the skin. As the number of melanophores remains constant across both blotched and wild-type fish, the differences in patterning do not appear to be due to variations in melanophore density but rather differences in migration behavior and timing. These observations suggest the hypothesis that the tissue environment plays a role in pigment pattern formation by modifying melanophore or precursor cell migration. Despite this, the molecular, developmental, and evolutionary mechanisms underlying the blotched phenotype remain unclear. To address this, we tracked melanophore behavior throughout development and examined key spatial gene expression differences to understand the observed differences in melanophores in these morphs.

Materials and methods: To investigate the developmental basis of the blotched phenotype in *H. chilotes*, we conducted comparative analysis of pigment pattern formation in blotched and wild-type morphs. Fish of both color morphs were imaged daily throughout embryonic development to track the timing of melanophore emergence and the cells migratory routes. Individual melanophores were counted and their migration paths were analyzed to assess speed and distance traveled across

development. To complement this, we used gene expression analysis with RNAscope on sectioned blotched and wild-type embryos. A panel of probes was designed for RNAscope to target pigment cell markers and candidate genes previously implicated in the blotched phenotype based on the literature and scRNA-seq analysis within our group. RNAscope data were used to compare regional gene expression between the blotched area and corresponding region in wild-type fish.

Results: Melanophore number and timing of initial emergence were similar between the morphs, indicating that the blotched phenotype is not due to differences in melanophore proliferation. However, melanophores in the blotched pattern migrated both faster and farther compared to their wild-type conspecifics. RNAscope showed spatially localized differences in gene expression between the blotched and wild-type morphs. The receptor tyrosine kinase gene *erbb3b*, showed elevated expression in tissue surrounding the blotch region compared to the corresponding wild-type regions. This gene has known roles in neural crest cell migration and development of subsequent pigment cells as well as being involved in establishing, maintaining, or recruiting the precursors of melanophores and might therefore be a crucial gene underlying this phenotype.

Conclusions: Our findings suggest that the blotched pigmentation phenotype in *H. chilotes* is not driven by changes in melanophore number, but by changed migration behavior, likely driven by expression differences in the migratory environment. Elevated expression of gene *erbb3b*, within the blotch region suggests that the local microenvironment or cell non-autonomous cues are impacting melanophore behavior. This contributes to the understanding of how gene expression variation and migratory behavior shape pigment pattern development and diversity.



Comparative repeatome evolution in diploid and allotetraploid *Trifolium* (clover)

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POSTER SESSION 2 - **P02.295**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Transposable elements (TEs) are major drivers of genome evolution in plants, contributing to genome size expansion, structural reorganisation, and subgenome asymmetry in polyploids. Comparative studies of TE dynamics across species are limited, particularly in non-model genera. However, with the increasing availability of high-quality genome assemblies, it is now possible to investigate repeatome evolution at a broader phylogenetic scale. The genus *Trifolium* (clover) contains species of ecological, agricultural, and cultural significance, including a number of allopolyploids. Here we use eight high-quality genome assemblies from *Trifolium* species — six diploids and two allotetraploids (*T. repens* and *T. dubium*) — to investigate the role of TEs in genome structure and post-polyploid evolution across the genus.

We present a comparative analysis of TE content across the genus, using a pan-genus TE library to annotate and compare repeat landscapes. Focusing on long terminal repeat retrotransposons (LTR-RTs), we look at insertion age distributions, TE turnover and potential TE-driven structural variation. In allotetraploid species, we partition chromosomes into subgenomes and compare LTR-RT content and accumulation between homoeologous chromosome sets.

Our results reveal lineage-specific variation in LTR-RT proliferation, as well as subgenome-biased retention patterns suggestive of divergent evolutionary trajectories following allopolyploidisation. Together, these findings highlight the contribution of LTR-RTs to genome structure and diversification in *Trifolium*, and provide insight into how TEs and polyploidy jointly shape plant genome evolution.



Diet evolution and genomic adaptations to sugar-feeding in the bat family Phyllostomidae

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POSTER SESSION 2 - P02.375, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Adaptive radiation is an important mechanism of biodiversification, and yet its evolutionary preconditions and genomic underpinnings are not fully understood. The adaptive radiation of New World leaf-nosed bats (family Phyllostomidae) is accompanied by diet diversification including arthropods, blood, terrestrial vertebrates, nectar, and fruits. However, when and how dietary transitions occurred in Phyllostomidae remain unclear. Here we test the hypotheses of ancestral omnivory (insect and fruit feeding) versus insectivory in Phyllostomidae (141 species) using improved trait reconstructions based on multi-response phylogenetic threshold models, while explicitly accounting for phylogenetic uncertainty. In addition, empowered by over 30 high-quality chromosome-level genomes of phyllostomid bats and high-accuracy annotation from TOGA (Tool to infer Orthologs from Genome Alignments), we identify genomic underpinnings of phyllostomid dietary transitions, with a focus on adaptations to fruit and nectar feeding. Our phylogenetic

reconstructions indicate early evolved complementary fruit feeding in the most recent common ancestor of Phyllostomidae, supporting the ancestral omnivory hypothesis. Reconstructions of all 21 bat families (621 species) further reveal independent evolution of complementary fruit feeding in four families, but only two (Phyllostomidae and Pteropodidae) evolved predominant/strict fruit feeding and high species diversity. Therefore, our results infer that ancestral generalism (i.e., omnivory) may be a precondition but does not necessarily lead to adaptive radiation which also requires subsequent adaptations and niche partitioning. Preliminary analyses using TOGA-inferred gene orthology show genomic signals of metabolic adaptations to predominant fruit and nectar feeding in phyllostomid bats. Our study presents a comprehensive picture of dietary evolution in bats and genomic adaptations to fruit and nectar feeding in Phyllostomidae, which informs the evolution and genomic bases of adaptive radiation.



Dissecting genes of color pattern in birds and their role in maintaining species boundaries in a moving hybrid zone

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POSTER SESSION 2 – **P02.035**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Sexual selection is a crucial factor in initiating speciation through behavioral isolation, potentially playing a more significant role than other types of barriers. Because sexual signals are often phenotypes composed of multiple individual traits (e.g. multiple color patches in color pattern), the genetic architecture of such composite sexual traits determines the strength of selection necessary to maintain species boundaries over time. For example, stronger selection is expected to be required to maintain composite sexual phenotypes when the underlying loci are distributed across the genome and are only functionally linked, rather than physically linked. Additive and epistatic interactions among loci will further modulate the required strength of sexual selection. However, the genomic architecture of composite phenotypes under sexual selection remains unclear, obscuring our understanding of the role of sexual selection initiating and maintaining species formation.

Here, we will utilize a moving hybrid zone between two closely-related songbirds, the black-headed bunting *Emberiza melanocephala* and the red-headed Bunting *E. bruniceps*. Males differ in plumage composed of different coloration patterns, with hybrids displaying various degrees of intermediate but also novel phenotypes. We capitalize on the fact that the hybrid zone is moving in response to environmental change, leading to more gene flow and hence greater homogenization of neutral genomic regions compared to stable hybrid zones. Using whole-genome sequencing data, we identify genomic regions that form barriers to gene flow by inferring geographic clines for several coloration traits and underlying genomic loci. We expect phenotypic clines to be steeper than the average genetic cline, indicating restricted introgression of phenotypic traits. Next, we will investigate the genetic architecture of

composite male coloration by identifying genomic and phenotypic clines that share a common center and width, presumably shaped by behavioral incompatibilities. Since the hybrid zone might not show a clear spatial clinal variation, we will infer genomic clines by comparing allele frequencies across the hybrid index, expecting steeper clines for barrier loci since most hybrid classes will only exhibit a single parental allele. To test whether the regions that act as barriers to gene flow correspond to those associated with plumage coloration, we will conduct a genome-wide association study (GWAS) and test for an additive or epistatic nature of phenotypes.

Finally, to understand how the genetic architecture of color patterns influences the trajectory of speciation, we will incorporate museum specimens sampled 50 years ago (~25 generations). We test whether barrier loci exhibit significantly less change in F_{st} than presumed neutral regions and whether functional and physical linkage decreased over time. To assess how the hybrid zone has shifted, we will compare the steepness, shape and center of geographic clines in both phenotypic and genomic traits between historical and contemporary samples.

We aim to show the unique value of moving hybrid zones to unravel the genomic architecture of complex traits and to understand how several generations of recombination can lead to novel sexual phenotypes. Assessing linkage relationships between barrier genes allows us to further determine the strength of selection needed to allow species to persist despite gene flow. As processes such as climate and land-use change are expected to lead to an increase in secondary contact zones, understanding these dynamics may be of greater importance in the future.



Distinct dynamics in a key herbivore detoxification gene family reveal mechanisms of convergent host plant shifts

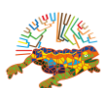
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POSTER SESSION 2 - P02.249, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The evolutionary success of herbivorous insects is thought to be due to adaptation to novel chemical defenses in host plants, but the genetic architecture that enables host plant transitions is not well understood. Convergent host plant shifts, where two or more related lineages independently colonize the same host plants, present a unique opportunity to associate genetic changes with dietary shifts. Pierinae butterflies are a diverse sub-family that rapidly diverged after the clade shifted to feed on Brassicales plants, which produce defensive glucosinolate secondary metabolites. Pierinae butterflies evolved nitrile specifier proteins (NSPs), a novel adaptation allowing them to neutralize toxic glucosinolate defenses characteristic of their host plants. Within Pierinae, multiple lineages have independently colonized Brassicaceae, a Brassicales lineage with a diverse and complex glucosinolate repertoire. This replicated ecological transition allows us to investigate the genetic

architecture of host plant adaptation through the lens of convergent evolution. In this study, we leveraged new and publicly available high-quality genomes to perform a comprehensive comparative genomic analysis to link patterns of gene family evolution to shifts in host plant usage. We identified distinct NSP-like gene family dynamics in two Pierinae lineages that independently adapted to chemically advanced Brassicaceae plants. We then demonstrated lineage-specific transcriptional responses of NSP-like genes to divergent glucosinolate profiles, suggesting subfunctionalization in some lineages but not others. Finally, we are developing a novel transgenic *Drosophila*-based functional assay to validate NSP's detoxification capacity. Our findings illustrate the genetic complexity underlying convergent evolution and provide insights into the molecular dynamics that drive coevolutionary interactions.



Distinct functional effects of individual nucleotide variants in Eip75B on life history adaptation in the fruit fly

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POSTER SESSION 2 - P02.334, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Advances in genome sequencing technology in the past decades have provided powerful tools to associate genetic variants with complex phenotypes that drive evolutionary adaptation. Our understanding of the functional impact of individual nucleotide variants (single nucleotide polymorphisms; SNPs) remains limited, however, as the vast majority of candidate variants from association studies awaits functional validation. Validation is critical to distinguish causal from neutral variants, and to determine how many and how strongly individual nucleotides influence complex phenotypes.

We investigated a set of three SNPs located in different regions of the nuclear hormone

receptor and PPAR γ -homolog Ecdysone induced protein 75B (Eip75B) that had previously been associated with the experimental evolution of ageing and life history in the fruit fly, *Drosophila melanogaster*. By performing genomic editing with the precision of a single nucleotide, using CRISPR/Cas9, we investigated the phenotypic effects of these intronic SNPs. These experiments revealed unique and significant effects of individual SNPs on fecundity, egg-to-adult-viability and starvation resistance, which emphasizes the importance of genetic tests at the level of individual SNPs to understand the genetic architecture of complex fitness-related traits.



Ecological shifts and genomic architecture divergence drive sexually selected weapon size reduction and reproductive isolation in island populations of rhinoceros beetles

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POSTER SESSION 2 - **P02.260**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Exaggerated weapons of sexual selection often diverge more rapidly and dramatically than other body parts, suggesting that relevant agents of selection may be discernible in contemporary populations. Because these weapons are deployed against the weapons of conspecific rivals, mate competition can generate consistent and intense directional selection for elaborations to weapon form that improve contest outcome.

In the Japanese rhinoceros beetle (*Trypoxylus dichotomus*), males harbour a characteristic “pitchfork” horn that can account for up to 1/3 of their body length. As in other holometabolous insects, adult phenotypes in beetles are shaped during a brief developmental window—the pre-pupal stage—through precise modulation of gene expression. Males with the longest horns are more successful in combat, better able to defend rare sap flows on tree trunks, and mate with more females. Yet, several island populations have independently evolved shorter horns. Mating trials done in our lab revealed that these island populations are reproductively isolated from mainland populations.

To test whether structural differences in genome architecture linked to weapon size difference contribute to the establishment and maintenance of reproductive barriers, we identified sister population pairs that differ strikingly in horn length. Leveraging the new chromosome-level genome assembly, we integrated multiple omics approaches—whole-genome sequencing to assess demographic history, PoolSeq to examine genome architecture, and RNAseq to

analyse gene expression in developing horn tissue—alongside three years of field-based fitness measurements and experimental crosses between short- and long-horned populations.

From proliferating horn tissues of different pairs of sister populations, we found that the exaggerated horn length requires an increased expression of the genes involved in sexual dimorphism, coupled with an alteration in cuticle structure for increased growth. But surprisingly, those genes didn’t show signature of selection and did not fall into low combining regions along the genome among populations. Instead, we found that the 50 genes falling within these outlier regions play a major role in wood digestion (insulin pathway, immune system). These genetic findings were supported by our field observations: island populations have shifted host trees, transitioning from hard-barked to soft-barked species that they can carve by themselves. This ecological change has major implications for male reproductive success. The increased abundance and dispersion of feeding territories reduces the likelihood that any one territory is visited by a female, thereby limiting the advantage of horn-based male-male competition as we measured during fieldwork.

While our results further understanding of the genetic underpinnings of sexually selected weapon evolution, they also highlight an overarching point: components of the social environment—such as the structure of mate competition—can evolve in response to ecological changes and broader contextual factors should never be overlooked.



Evolution and divergent genetic mechanisms of annual and perennial *Hordeum* species

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POSTER SESSION 2 - **P02.215**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Life-history strategies in angiosperms, notably the contrast between annuality and perenniality, underpin key ecological and evolutionary adaptations. Despite their importance, the genomic basis and macroevolutionary trajectories distinguishing these strategies remain poorly characterized across many groups. The grass genus *Hordeum* (barley) comprises both annual and perennial species and exhibits multiple independent transitions between them. Here, we present a comparative phylogenomic investigation of *Hordeum* aimed at (1) resolving phylogenetic relationships and detecting interspecies hybridization, (2) comparing climatic niches of annuals and perennials, and (3) exploring genetic mechanisms (copy number variation, differential expression, divergent signatures of selection) that differentiate annual and perennial *Hordeum* species.

We generated and assembled transcriptomes for 82 accessions – encompassing all 24 diploid *Hordeum* species and two outgroups – and integrated these with 24 recently published reference genomes. From this dataset, we extracted a suite of single-copy orthologs to reconstruct phylogenetic relationships, calibrate divergence times, and infer ancestral life-history regimes. To uncover inter-species hybridization, we applied phylogenetic network modelling alongside D-statistic tests. Leveraging over 2,500 georeferenced occurrence records merged with high-resolution climatic and land-cover data, we conducted comparative analyses of niche occupation. Finally, we performed genome-scale scans for divergent selection signatures between annual and perennial *Hordeum* species, contrasted expression patterns, and quantified gene copy number variation to identify genetic correlates of annual versus perennial strategies.

Phylogenetic analyses yielded a highly resolved and congruent topology across multiple inference frameworks, revealing that the ancestral *Hordeum* was annual and that perenniality evolved independently on at least two occasions – followed by a single reversion to annuality – over the genus' ten-million-year history. Network modelling and D-statistic tests uncovered four robust instances of interspecific gene flow, three of which align with long-distance dispersal between Eurasia and the Americas. These findings highlighted the contribution

of hybridization to *Hordeum* diversification and established a framework for comparative phylogenetic investigations into the ecological and genetic underpinnings of life-history strategies.

Ecological niche analyses showed that perennial *Hordeum* species occupy a substantially broader climatic space than annuals. Perennials extend into regions with greater variation in temperature seasonality, whereas annuals are largely confined to areas with more extreme conditions during their growing season. Habitat assessments further indicated that annuals more frequently colonize areas with low ecological predictability like human-disturbed environments. Together, these findings suggest that annuality in *Hordeum* may confer an adaptive advantage under conditions of environmental unpredictability, consistent with life-history theory and previous findings in other plant taxa.

Analyses of divergent genetic mechanisms uncovered 92 genes with divergent signatures of selection, implicating functions in metabolism, developmental regulation, and stress response. Expression profiling across transcriptomes identified 41 genes consistently up- or down-regulated in annuals versus perennials, with annuals showing enhanced expression of protein-turnover and growth-acceleration pathways, and perennials upregulating genes linked to resource allocation and stress tolerance. Gene-family analyses detected multiple families expanded in perennials, many of them transposon-related. Overall, our analysis of divergent genetic mechanisms in annual and perennial *Hordeum* species implicated genes spanning diverse biological functions in all three analysed genetic mechanisms. This diversity in candidate genes suggests that multiple, interconnected biological processes collectively contribute to the contrasting life-history strategies in *Hordeum*.

Our comparative phylogenomic analyses pinpointed key candidate genes and potentially adaptive niche shifts, laying the groundwork for future studies to deepen our understanding of the genetic basis of life-history strategies and their role in plant evolution.



Evolution of Transposable Elements in the Swedish Sand Lizard, *Lacerta agilis*

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POSTER SESSION 2 - **P02.377**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Transposable Elements (TE's) or transposons are DNA sequences that can move independently inside eukaryotic genomes using self-coded enzymes. TE's are classified into Classes, Orders, and Superfamilies based on their transposition intermediates, mechanism of transposition, and phylogeny. Transposons can cause large-scale genomic rearrangements, inactivate genes, and can up- or down-regulate genes, among a plethora of other genomic effects.

So far, TE studies have been biased towards mammals and birds, while reptiles remain understudied. This is especially significant because in squamate reptiles (i.e. lizards and snakes), genome size is not tightly-linked to TE-content. Moreover, squamate genomes have young and actively-transposing TE families. Moreover, there is evidence to suggest that squamates have common mechanisms constraining their genomes. Thus, population-level studies of TE families are required, as they would help us understand important patterns in TE aggregation, deletion, and domestication. Therefore, it is important to carry out these studies in additional squamate species.

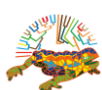
The *Lacerta agilis* species complex occupies a wide range, from the UK in the west to Lake Baikal in the east, with the Swedish population being the northernmost in Europe. The Swedish sand lizard populations are fragmented, and often isolated from one another, are classified as vulnerable and protected under a species management plan. It is vital that we know as much about this species as possible, so that we may conserve it. So, we are studying TE's in the sand lizard genome.

Materials and methods: In order to characterise the TE content of this genome, we ran RepeatModeler2 (RM2) on the reference genome,

and then manually curated the TE families found by RM2. The curated TE dataset was then used to find how many members of each TE family are present, and their genomic locations, both in the reference genome, and a newly-generated genome, using RepeatMasker. This allowed us to construct a Repeat Landscape for the genomes, and find the ages of the TE families. Then, phylogenies were constructed for various TE families, including members of these TE families in other Lacertidae. For one TE Superfamily, LTR transposons, we estimated the timing of each insertion by calculating the divergence between their 5' and 3' LTR's.

Results: We found that repetitive elements made up 43.19% of the sand lizard genome, 42% of which RM2 was able to classify into known Orders such as LINE, LTR, and DNA transposon. The largest Order of TE's was LINE, which contained 18% of the TE families found. Out of the families found by RM2, 65 were prioritized for manual curation. Among these high-priority families, the largest Order was LTR, at 68%, and among LTR's, most of the TE families were from the Superfamilies Copia (52%) and Gypsy (30%). More information about the different types of TE's, as well as their proportion within the genome can be found in the Repeat Landscape.

Conclusions: Our results will help us understand what effects TE's have had on the sand lizard genome, such as large-scale, TE-mediated genomic rearrangements or changes in gene regulation. We will use population-level data to find differences in TE accumulation, deletion, and domestication between Swedish sand lizard populations. This genome- and population-level TE study will add to the body of work on squamate TE's, and help elucidate the common mechanisms that constrain squamate genomes.



Evolutionary analysis of diet adaptations in anatomically modern humans using ancient genomes

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POSTER SESSION 2 - **P02.306**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Anatomically modern humans rapidly adapted to a wide range of environments following the Out of Africa event, undergoing significant evolutionary changes within a relatively short timeframe. This swift adaptation was driven by a combination of cultural and biological factors, notably including major dietary shifts associated with cultural transitions over the past millennia. These shifts are hypothesized to have exerted selective pressures on the human genome, with the FADS gene family serving as a key example. Diet-related traits represent a complex network of behavioral, metabolic, and anthropometric phenotypes, often influenced by numerous pleiotropic genetic factors. Previous efforts to infer and detect polygenic selection often faced limited statistical power due to weak signals, confounding demographic histories, or stabilizing selection effects. However, the increasing availability of ancient DNA (aDNA) datasets offers new opportunities to investigate the evolutionary dynamics of these traits through direct temporal evidence.

Materials and Methods: We integrated Genome-Wide Association Studies (GWAS)

with imputed aDNA data from 946 unrelated European individuals to investigate the evolutionary history of diet-associated traits. A custom pipeline was developed to curate relevant GWAS datasets, assess statistical power, and ensure quality control. After filtering and curation, remaining complex traits related to diet were clustered based on their shared genetic architecture using a genetic algorithm. Moreover, genetic interactions among traits were further explored using deep learning models. Temporal evolutionary trends were then analyzed using the ancient genomes. Genetic algorithms were further used to address additional questions of interests, such as in which time periods changes in selective pressures potentially occurred or if significant differences of temporal trends across distinct geographical regions are detected.

Conclusions: Our findings suggest a dynamic interplay of evolutionary forces driven by pleiotropy that have shaped human dietary adaptations over the past 30,000 years. In addition, tailored machine learning approaches offer powerful tools to advance the study of complex trait evolution.



Evolving inversions: the genomic architecture of parallel snail ecotypes in Sweden and France

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POSTER SESSION 2 - P02.281, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Structural Variants (SVs) that reduce recombination in heterokaryotype individuals have been shown to facilitate local adaptation by sheltering locally favourable genetic variants from gene flow. Hybrid zones established along environmental gradients provide a particularly powerful setup to study the role of SVs in local adaptation and ecological isolation, but with the limit that other reproductive barriers may coincide with environmental transitions. Thus, disentangling the role of SVs in local adaptation and ecological isolation from different types of barriers to gene exchange remains a major challenge. One way to address this issue is to establish links between the genomic variation and the phenotypic traits involved in ecological adaptation and ecological barrier mechanisms in an attempt to identify genomic regions containing barrier loci, and the selective forces involved. This approach should be particularly interesting when repeated across independent environmental gradients, as it may reveal differences in how SVs influence the evolution of ecotypes.

To this end, we used the marine snail *Littorina fabalis* that segregates locally into a dwarf and a large ecotype over wave exposure gradients in north-western European shores. Previous work focusing on a local hybrid zone in Sweden revealed strong genetic differentiation between ecotypes, concentrated in 12 putative chromosomal inversions. Here we aimed to test if similar genomic architectures (e.g., the same inversions) contribute to adaptation in geographically separated parallel environmental gradients, and more specifically through their role in the determinism of adaptive phenotypes.

Material and Methods: We compared the phenotypic and genetic composition of two hybrid zones, one from Sweden and one from France, both distributed over wave exposure gradients. Using whole genome sequencing data of 305 individuals, we identified polymorphic SVs along the transects and study the role of these SVs in the parallel genetic differentiation between ecotypes. Using Genome Wide Association Studies (GWASs), we investigated the genetic bases of phenotypic traits (shell size and colour) thought to be differentially adaptive in the two locations.

Results: Most of the genetic differences between ecotypes are clustered in 15 shared inversions that showed overlapping arrangement clines in both hybrid zones. However, a high number of SNPs within the inversions showed clines specific to one hybrid zone, and also reversed cline patterns between zones. Interestingly, we found the shell size cline between dwarf and large ecotypes to be reversed in France compared to in Sweden. This shell size pattern was detected by GWAS to be significantly associated with genomic regions within inversions, but only in Sweden. We also observed a strong association between shell colour and a large genomic region on an inversion in France, whereas nearly all Swedish snails were of the same colour.

Conclusion: Our results show that while many of the same inversions contributed to ecotype differences and local adaptation in two distant geographic regions, the content of the inversion arrangements was fine-tuned under the site-specific environmental conditions.



Functional analysis of yellow gene's cis-regulation in *Pieris canidia* butterflies

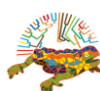
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POSTER SESSION 2 - **P02.382**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The wide diversity of color patterns in butterflies presents a fascinating biological system to advance our knowledge on color pattern evolution. A big part of how insect color patterns evolve has been linked to changes in the regulation of pigmentation genes. While the same pigment can be produced in different color patches, pigmentation patterns can diversify through pigment-producing genes gaining modularity in their regulation. This is achieved by the evolution of different cis-regulatory elements (CREs) controlling a subset of the gene's expression. The melanin gene, *yellow*, is a model example of this type of modularity in *Drosophila*, having distinct CREs regulating its expression in different body parts, that can independently evolve to create divergent melanin patterns across species. However, our current understanding of the gene's modular regulation in *Drosophila* may not be representative of other insect taxa, such as butterflies.

Here, I provide insights into *yellow*'s regulation in the Indian cabbage white butterfly, *Pieris canidia*. Using CRISPR/Cas9, I functionally validated a candidate CRE of *yellow* and observed its effects on melanization in adults of this species. Interestingly, removal of this CRE alone showed loss of melanization in multiple tissues across the body. The pleiotropic effect in this CRE in *P. canidia* appears to contrast with the tissue-specific nature of CREs in *Drosophila* and another Lepidopteran species *Bombyx mori*. Further sampling of more species within Lepidoptera, and outside of *Drosophila*, will allow a better understanding of the ancestral state of *yellow*'s CREs and their evolution. As the second Lepidopteran species studied, my findings highlight the value of studying gene regulation beyond *Drosophila* species to provide new insights into the evolution of gene regulatory architecture.



Gene copy number variations in Brassicaceae

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POSTER SESSION 2 - P02.248, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Gene copy number variations (gCNVs) are prevalent in eukaryotic genomes, perhaps even more so in plant genomes. In contrast to single nucleotide polymorphisms (SNPs) and balanced structural variants such as inversions, gCNVs are unbalanced: they cause the dosage of a gene to vary between individuals in a population. Their role in evolution and impact on phenotypic traits was suggested already in the early 20th century. However, in part due to past technological limitations and in contrast to SNP based studies, gCNVs are understudied, meaning there is still much to learn about their evolutionary importance. In recent years, technological advances such as long-read sequencing and a large number of statistical methods for detection of gCNVs have facilitated their study. Recent studies have shown gCNVs implicated in a wide variety of phenomena such as disease, local adaptation and the heritability of complex traits. Across plants, repeated events of mating system shifts, hybridisation and polyploidisation have led to a large variation

in genome sizes, likely contributing to the prevalence of gCNVs. Selfing species suffer from a drastic reduction in effective population size, meaning that a shift in mating system affects the efficacy of purifying selection. To explore whether gCNVs are associated with plant mating system shifts, we grew and sampled individuals of 15 selfing and 10 outcrossing Brassicaceae species. Using statistical methods on whole genome short-read sequencing data, we then identified gCNVs in these species. In the small diploid selfing Brassicaceae species *Capsella rubella*, or pink shepherd's-purse, we validated the statistical quantification using qPCR on a few of the target genes we identified in the same accessions using whole genome short-read sequencing data. In this talk I will be sharing insights from exploring gCNVs across the studied Brassicaceae species, as well as our findings when comparing statistical methods for CNV detection to more direct molecular techniques.



Genetic and molecular dissection of heteranthery in Solanaceae

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POSTER SESSION 2 - **P02.246**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Solanum is a large and diverse genus in the Solanaceae family. Known for containing several major crops -tomato, eggplant and potato- it also presents high morphological diversity and, recently, genetic and genomic resources have been developed for several species within the clade. Inside the genus diversity, we can find species with different flower morphology. While most of the species present radially symmetrical flowers with equal-length stamens, like tomato or Solanum sisymbriifolium, bilaterally symmetrical flowers with heteromorphic stamens have independently emerged at least 14 times, as in Solanum citrullifolium. Heteromorphic stamens, a trait known as heteranthery, are also present in other plant clades, usually associated with differential gene expression of paralogs. Understanding how heteranthery is genetically controlled can reveal how selective pressures and genetic architecture drive the evolution of novel morphological traits.

Materials And Methods: We are following a comparative approach, with S. sisymbriifolium (non-heterantherous) and S. citrullifolium (heterantherous) as our main system, but also accounting for other transitions to heteranthery in the Solanum genus. Through a multidisciplinary lens, we are applying a diverse array of transcriptomics, forward genetics -inter-specific populations and mutagenesis-, reverse genetics -CRISPR/Cas9 gene editing and complementation assays-, genomics and phylogenomics.

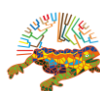
Results: By observing meristem and flower development in both species, we concluded the change in symmetry takes place in early stages of flower formation, where, in S. citrullifolium, a bilaterally symmetrical floral bud arises from a radially symmetrical floral meristem. In a detailed unbiased expression analysis in both species in developing meristems, as well as floral buds, we found several candidate genes with interesting expression dynamics both temporal and spatially. Of those candidate genes, TCP7

arose as the most promising one, as it presents a clear top-bottom differential expression in the flower bud of the heterantherous species and it is also the ortholog -together with its paralog TCP26- of snapdragon CYCLOIDEA and DICHOTOMA, well-known regulators of flower symmetry. As TCP26 does not present that promising expression profile, we hypothesize TCP7 has evolved a new role in controlling flower symmetry in Solanum. This gene and other candidates are being studied using reverse genetics -CRISPR and complementation assays- trying to unravel their role during meristem and flower development.

Additionally, we are following a forward genetics approach. Through a mutagenesis experiment in S. citrullifolium we have found several mutants affecting heteranthery, whose mapping and cloning will allow us to find new players in the development of this morphological novelty.

On top of that, we are also generating inter-specific mapping populations in other heterantherous Solanum clades. This approach, together with the generation of high-quality genomes of a diverse set of species and the subsequent phylogenomic studies, will allow us to understand how heteranthery has independently evolved in the genus.

Conclusion: This study provides insight into the genetic mechanisms underpinning the independent evolution of heteranthery in Solanum. By combining forward genetics, genomics, transcriptomics, and reverse genetics, we are identifying several candidate genes regulating floral symmetry and heteranthery in S. citrullifolium and other species. Understanding this genetic control not only clarifies how morphological diversity arises through gene duplication and neo-functionalization but also illuminates broader principles of plant developmental evolution driven by genetic architecture and selective pressure.



Genome Architecture-Aware Investigation of Earthworm Terrestrialization Amidst Extensive Genomic Reshaping, Rearrangements and Polyploidy

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POSTER SESSION 2 - P02.284, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Recent studies have shown that extensive chromosomal rearrangements triggered by a chromosomal shattering event profoundly reshaped the genome architecture in clitellate annelids. This led not only to a complete breakdown of chromosomal homology with marine annelids and all other metazoans, but also to a cascade of otherwise rare massive events that fully reshaped the clitellate genome architecture, such as whole genome duplications (WGD), large rearrangements between closely related lineages and chromosome fusion and fission events. These extensive dynamics and their potential role facilitating ecological adaptation are largely unexplored. In this study, we present the first chromosome-level genome assembly of a cave-dwelling, freshwater annelid species from the closest sister lineage to the terrestrial Crassicitellata (earthworms), offering new insights into the genomic shifts potentially associated with terrestrial adaptation. As in other clitellates, the Hox genes in this freshwater annelid genome lack canonical organization and are highly rearranged across different chromosomes, with a unique genomic architecture that potentially facilitated Hox gene long-range

interactions. Interestingly, although this species is more closely related evolutionarily to earthworms (fully terrestrial), its Hox gene complement more closely resembles that of freshwater leeches, with high levels of Hox gene loss. We developed a novel genome architecture-aware approach to disentangle the history of polyploidy and its potential role in earthworm terrestrialization, bypassing the constraints associated with the current methodologies, which largely rely on syntenic conservation. We revealed a complex pattern of WGD that has been obscured by the extensive chromosomal rearrangements and substantial differential gene loss. The polyploid events shared between this freshwater annelid and its terrestrial relatives exhibit signatures of asynchronous rediploidization, which may have contributed to earthworm divergent phenotypic and ecological trajectories. Our findings shed new light on the genomic foundations of annelid terrestrialization and offer a novel framework for disentangling WGD in the context of the unprecedented genome scrambling, illuminating how these drastic shifts in genomic and chromosomal architecture have driven lineage-specific genomic innovations in Clitellata.



Genome Size: Did Flight Loss Relax Genome Size Constraints in Birds?

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POSTER SESSION 2 - P02.267, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Genome size and its role in genome evolution has long been debated in evolutionary biology, with different trends emerging across taxonomic groups. Birds—compared to mammals and reptiles—are known to have a more conservative range of genome sizes, often attributed to the energetic demands of flight and the associated need for reduced cell size. In this study, we investigate whether flight loss in birds has released the genome size constraint observed in their volant relatives. To address this question, we constructed a dataset comprising short-read sequencing data of 30 bird species (13 taxonomic families) obtained from the NCBI database, including 15 flightless and 15 flighted species, representing 25% of extant flightless birds. Genome size estimations were performed using GenomeScope, revealing that ratites

and penguins possess significantly larger genome sizes than “other” flightless birds (7 species) and their flying relatives. The “other” flightless birds did not deviate in genome size compared to their flying relatives. Regressions further indicate that body mass, together with flight ability, explain 48% of the variance in genome size. Based on our preliminary results, we plan to broaden our analyses by incorporating extreme body mass comparisons and applying models that account for phylogenetic relationships. We aim to extend this analysis across the broader avian phylogeny, with a focus on species that evolved flightlessness more recently, to better understand the evolutionary relationship between genome size and flight capability.



Genomic bases of a pollinator-driven speciation in sexually deceptive orchids of the *Ophrys insectifera* clade

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POSTER SESSION 2 - **P02.378**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Sexually deceptive orchids of the genus *Ophrys* experience a striking evolutionary radiation in the Mediterranean Basin, relying on highly specific pollination systems. Their flowers mimic the appearance, texture, and scent of female insects to attract males, inducing pseudocopulation and ensuring pollination. This unique interaction has driven an exceptionally rapid diversification - up to 350 species in less than 5 million years. However, this tight dependence on specific pollinators also makes *Ophrys* species particularly vulnerable to environmental modifications. In this context, understanding the genomic basis of pollinator specificity and its role in speciation is both a fundamental and conservation-relevant question.

Material and Methods: We investigated three species within the *Ophrys insectifera* clade (*O. aymoninii*, *O. insectifera*, and *O. subinsectifera*) whose taxonomic boundaries remain debated. To uncover the genomic mechanisms underlying pollinator-driven divergence, we integrated (i) population genomic data from >11,000 RAD-seq-like approach derived markers, which offers a cost-effective alternative to whole-genome sequencing given the large genome size of *Ophrys* (>5Gbp) and the challenges of cultivating these orchids under controlled conditions, (ii) transcriptomic profiling of floral tissues, and (iii) detailed floral phenotyping including morphology, scent (via GC-MS), and visual traits (via calibrated photography) - all performed on more than 200 individuals sampled across the distribution ranges.

In parallel, we conducted a 4-year individual-based monitoring of these same individuals to assess fitness proxies: pollinia removal (male success) and fruit set (female success), as well as plant survival and re-flowering. We used GWAS and Multi-Omics Factor Analysis (MOFA) to identify genomic regions associated with floral

traits involved in pollinator attraction and reproductive isolation.

Results: Genomic analyses confirmed the distinctiveness of the three species, supporting their recognition as separate evolutionary lineages. Patterns of gene flow revealed asymmetric introgression across the Pyrenees, indicating geographically structured reproductive isolation.

Floral scent analyses identified species-specific bouquets composed of volatiles mimicking pollinator sex pheromones, attracting distinct pollinator families. Visual and morphometric analyses showed strong trait matching between flower and pollinator morphology, along with color traits, such as a distinctive yellow labellum margin contributing to species discrimination.

GWAS and MOFA analyses revealed candidate genes such as *SAD2* (involved in volatile biosynthesis) and *FLS* (in flavonol pigment pathways), directly associated with interspecific variation in floral signals. These results illustrate how pollinator preferences shape the evolution of multiple integrated traits and their genomic underpinnings.

Ongoing analyses of long-term fitness monitoring aim to provide key insights into the ecological relevance of floral traits and the genomic regions associated with reproductive success.

Conclusion: Our study highlights the genomic bases by which specialized floral traits drive speciation in *Ophrys*. By combining genomics, transcriptomics, and precise floral phenotyping with long-term fitness monitoring, we provide a comprehensive understanding of how pollinator-mediated selection shapes species boundaries. These findings not only shed light on the mechanisms of plant diversification but also inform conservation strategies for taxa whose survival hinges on fragile plant-pollinator interactions in a changing world.



Genomic drivers of persimmon adaptive radiation in New Caledonia, a biodiversity hotspot

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POSTER SESSION 2 - P02.360, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Adaptive radiation, the rapid evolution of phenotypic and ecological diversity within a monophyletic lineage, is a major contributor to global biodiversity. While ecological opportunity has long been considered its primary driver, recent studies suggest that intrinsic genomic factors and changes to genome architecture, mediated by the activity of transposable elements (TEs) and the accumulation of structural variants (SVs), together with hybridization and demographic changes, may foster the evolutionary potential of lineages. These features can increase mutational input, modulate gene regulation, and promote the emergence of ecologically relevant traits. However, the relative contribution of genome dynamics to diversification remains poorly understood, especially in plants.

The genus *Diospyros* (Ebenaceae) comprises over 700 species globally, with at least 35 endemic to New Caledonia, a biodiversity hotspot with the highest level of plant endemism. The island's complex geological history has produced a diverse mosaic of contrasting substrates (ultramafic, calcareous, and sedimentary), steep elevation gradients, and distinct climatic zones within a limited area. These characteristics provide a natural laboratory to investigate how species respond to ecological heterogeneity. Remarkably, *Diospyros* colonized New Caledonia four times independently over the past ~20 million years, but only one lineage underwent a rapid adaptive radiation, giving rise to roughly 30 species that occupy nearly all ecological niches across the archipelago, except mangroves. The three other lineages remain species-poor (1–5 species each), with narrow ecological and geographic distributions. Notably, several species in the radiated clade thrive on ultramafic soils, which are characterized by low fertility, poor water retention, and high concentrations of toxic metals, conditions known to exert strong selective pressure on root physiology and metal detoxification mechanisms. Intriguingly, this radiated group exhibits a striking increase in genome size (~2×) compared to the non-radiating lineages, despite no change in ploidy,

suggesting major changes in genome architecture and TE activity.

To investigate how genome dynamics contributed to the diversification of New Caledonian *Diospyros*, we used a pangenomic approach to compare 27 newly assembled and annotated genomes (PacBio HiFi), about 500 resequenced accessions (Illumina), soil and leaf elemental content, and root and leaf transcriptomes from species adapted to contrasting soil types. We used Progressive Cactus to build a graph-based pan-genome for the radiated clade and GraffiTE to detect lineage-specific TE insertions. SVs were called using Sniffles2 and were integrated with gene annotations and transcriptomic profiles.

We find that the radiating clade shows elevated TE activity and extensive lineage-specific structural variation, including insertions, deletions, and inversions overlapping genes involved in abiotic stress response, metal detoxification, and root development. McDonald Kreitman tests suggest a faster evolution for several genes related to relevant abiotic stress. Gene expression data reveal environment-specific regulation in loci associated with metal transport and ion exchange in ultramafic-adapted species. Moreover, SV hotspots in proximity to differentially expressed genes and putative regulatory elements suggest potential roles in shaping expression divergence and adaptive phenotypes.

Our findings indicate that genome expansion and dynamic structural variation may have increased the evolutionary potential of the radiating *Diospyros* lineage, enabling the exploitation of diverse and challenging environments. These results support the hypothesis that shifts in genome architecture, particularly TE proliferation and SV-mediated regulatory innovation, can act as catalysts for diversification. By linking genomic processes to ecological transitions, this study contributes to a deeper understanding of how genetic architecture underpins adaptive radiation in plants.



Genomic impact of asexuality in oribatid mites

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POSTER SESSION 2 - **P02.196**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Sexual reproduction has been shown to be crucial for rapid adaptation to changing environments and to avoid deleterious mutations and evolutionary dead ends in many species. However, some animal species have survived in the absence of sexual reproduction by propagating parthenogenetically, yielding clonal offsprings. Oribatid mites have existed for millions of years, in some cases even over a hundred million, and are found on all continents in soil and freshwater. They include sexual and asexual species. The mechanisms underlying their transition to parthenogenesis, and the impact of this mode of reproduction on their genome, is unknown.

To investigate the consequences of such a persistent parthenogenetic mode of reproduction over large time scales, we collected a dataset of 11 genomes, including four sets of closely related sexual and asexual oribatid mite species. The genomes were assembled using PacBio HiFi, TELL-Seq and Hi-C reads, into highly contiguous sequences, and chromosome candidates for four species. The genome structures and compositions were compared to their sexual counterpart.

Chromosome-level assemblies of *Steganacarus magnus*, *Atropacarus*

striculus, *Platynothrus peltifer* and *Hermannia gibba* reveal a mostly conserved genome consisting of nine chromosomes with few rearrangements, except for the latter, which has eight. The 11 genomes also have a large set of shared orthologs, even though these species diverged over 200 million years ago. In addition, asexual species have remained diploid. Parthenogenetic species have strikingly smaller genome sizes than sexual species, correlating with decreased numbers of genes and transposable elements. However, asexual species generally exhibit larger proportions of horizontal gene transfers, in majority of bacterial origin, and around one third are of putative eukaryotic origin.

These results suggest that asexual oribatid mites may defy Muller's ratchet as successful parthenogens. The low content of transposable elements in asexual species could indicate a selection against their accumulation, as to avoid genomic instability. The higher number of horizontal gene transfers could act as a source of genetic diversity and adaptation in the absence of recombination. These findings provide insight into how these asexual lineages may have avoided an evolutionary dead end and managed to survive as strict parthenogens



Haplotype structure – an overlooked key factor shaping the genomic selection response

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POSTER SESSION 2 - **P02.374**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Genetic redundancy, a hallmark of polygenic adaptation, implies that new trait optima can be reached by frequency changes of a subset of contributing loci. Hence, the genomic response is often heterogeneous among replicate populations. However, even when many contributing loci segregate in a polymorphic population, linkage disequilibrium (LD) can constrain the genomic response by causing linked alleles respond jointly. Consequently, rather than individual SNPs, haplotype blocks with multiple selection targets are responding to selection. Although the concept of linked selection is well established, the extent to which LD shapes the genomic response to polygenic adaptation remains unclear.

Materials and Methods: To test the role of LD in shaping the genomic responses empirically, we used different sets of isofemale lines derived from the same natural *D. simulans* population. Because randomly sampled chromosomes differ in their linkage structures, we hypothesized that replicate populations founded from the same set of chromosomes would exhibit more similar genomic responses than those established from different founder sets. We tested this prediction of polygenic adaptation with experimental evolution.

From one *D. simulans* population we sampled three sets of isofemale lines to create three supergroups, and five replicates of each supergroup independently evolved in two temperature regimes: a hot fluctuating environment (28/18°C) and a hot constant environment (23°C). Pool-Seq data were collected every 10 generations up to generation 80.

Results: Consistent with the central role of haplotype structure for genomic selection responses, PCA clustered replicates, but clearly separated the three supergroups. The allele frequency changes were more strongly correlated within supergroups than between. Notably, this pattern persisted even across temperature regimes: differences between supergroups were more pronounced than differences between environment regimes. Additionally, forward simulations aligned with the empirical data further support our interpretation that LD plays a substantial role in driving the genomic response of polygenic adaptation.

Conclusion: The key role of LD in shaping the genomic selection response highlights the stochastic nature of adaptation from standing genetic variation and questions the expectation of parallel selection responses, even for adaptation to the same stressors.



Identification of genes associated with domestication by using three different statistics

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POSTER SESSION 2 - **P02.379**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Pig domestication took place 10,000 YBP and encompassed the selection of several traits linked to behavior (tameness), improvement of reproductive capacity, muscle composition, body size and coat color. Genomic regions under selection can be identified because they show shifts in the site frequency spectrum (SFS), decreased genetic variability, and high frequency of long haplotypes. Herewith, we have used 3 statistics (FST, XP-EHH and μ) to identify candidate selection signals potentially associated with domestication in European and Asian pig and wild boar populations.

Materials and methods: Whole genome sequences of 101 European wild boars (EUW), European pigs (EUD), Asian wild boars (ASW) and Asian pigs (ASD) were retrieved. Variant calling and phasing were performed with GATK and BEAGLE, respectively. After variant quality filtration, 57,034,815 SNPs were used for the detection of selective sweeps. FST and XP-EHH values were computed in 40-kb genomic windows by comparing wild boars against pigs in Europe and Asia (EUD vs. EUW & ASD vs. ASW). In domestic Asian and European pig populations, the composite μ statistic was calculated in sliding windows (SNPs > 50) with the RAiSD software. Candidate selective sweeps were defined based on the overlap of the genomic positions in the top 1% for FST and XP-EHH values and top 0.05% for μ values. Genes within putative selective sweeps were identified with Ensembl and levels of genetic variability, linkage disequilibrium (LD) and shifts in SFS were investigated with RAiSD.

Results: For FST, 1133 genomic windows showed extreme values in the Asian

comparison and 610 genes were found within these regions; while in the European contrast 537 genes were retrieved from 1133 genomic windows in the top 1%. For XP-EHH, in the Asian contrast we found 674 genomic regions with extreme values and 556 genes were identified, while in the European contrast we retrieved 609 genes from 676 regions in the top 1%. Finally, for the μ statistic, in the Asian population we identified 981 genes within 19,875 genomic regions displaying 0.05% top μ values, while for European populations we detected 1060 genes in 12,313 genomic regions. In the European analysis, 34 genes mapped to potential selective sweeps achieving top values for the 3 statistics, while in Asia we observed 39 genes. Examples of genes detected in this way were NR6A1, which is associated with the numbers of vertebrae in pigs and exhibited reduced genetic variability and shifts in the SFS based on the μ -statistic calculated with RAiSD. The melanocortin 1 receptor, involved in pigmentation, also displayed strong signatures of selection (high levels of LD, shifts in SFS and reduction of local variability), especially in its 3' upstream region. Moreover, genes related to spermatogenesis (GPR149), neurogenesis (SEMA3E) and brain function (SCN2A) also mapped to potential signatures of selection in this study.

Conclusions: Our results suggest that pig domestication involved a strong selection of genes related to key biological processes such as neural development, fertility, coat color and body size. Our sweep analysis based on 3 statistics gives robustness to find true signatures of selection link to pig domestication.



Linked selection of insertions and deletions in coding regions of the great tit genome

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POSTER SESSION 2 - P02.376, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Insertions and deletions (indels) are the most frequent structural variations and the second most common type of genetic variation in genomes, with the potential to affect multiple bases within one single mutational event. However, the genetic footprints of selective forces on indel mutations, such as hard sweeps and soft sweeps, remain unknown and challenging due to technical limitations. In this study, we calculated the non-synonymous substitution rate (dN), the synonymous substitution rate (dS), and the ratio of

nonsynonymous to synonymous substitutions (ω) in the regions surrounding indels to evaluate their genetic footprints in the great tit genome. Our findings reveal that the regions surrounding indels exhibit low dS values, suggesting that synonymous mutations in these regions appear at lower rates or that they are under selection. Furthermore, the strength of selection on these indel surrounding regions not only varies between insertions and deletions but is also significantly influenced by indel size.



Modeling the co-evolution of genetic individual condition and condition-dependent traits involved in sexual selection

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POSTER SESSION 2 - **P02.307**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The expression level of many traits depends on an individual's condition, and this condition-dependence is particularly pronounced for traits involved in mate choice. In sexual selection theory, female preferences for elaborate male ornaments can evolve and be maintained when these ornaments reliably reflect male condition, thereby providing direct or indirect fitness benefits to choosy females. For such signaling systems to be stable, variation in male condition must persist in the population.

Most theoretical models assume that variation in condition is fixed — often maintained through high mutation rates — and do not allow condition to evolve dynamically. However, when condition has a genetic basis, it can co-evolve with the condition-dependent traits it influences, thereby modifying the selective landscape for both male ornaments and female preferences.

To investigate the co-evolution of genetic variation in condition with condition-

dependent sexual traits, this project extends classical models by introducing two key components. First, female preferences are modeled as condition-dependent, similarly to male ornaments, based on empirical evidence that females in better condition tend to express stronger mate preferences, likely due to condition-specific costs of choosiness. Second, the genetic architecture of condition is explicitly modeled, and several scenarios are compared — including condition determined by many loci of small effect, a single major locus, or loci with sexually antagonistic effects — to identify which architectures are most likely to support the evolution of female preferences through indirect genetic benefits. These predictions will be especially useful to understand the role of sexual selection in population health.

This work marks the beginning of a PhD project that started in December 2024. While no results are available at this stage, the first theoretical findings will be ready to present at the time of the conference.



Pangenomic variations and differentiation between the two African malaria vector sister species *Anopheles gambiae* and *Anopheles coluzzii*

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POSTER SESSION 2 - **P02.386**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Structural variations (SVs) play pivotal roles in genome architecture, potentially involved in adaptation and speciation, yet they are often overlooked. Here, we delved into the pangenomic genomic landscape of SVs within *Anopheles gambiae* and *Anopheles coluzzii*, principal African malaria vectors. We used a "map-then-assemble" pangenomic strategy with short-read sequencing data, validated with samples for which short- and long-read data were available. Then we assessed pangenomic diversity and divergence in the two sister species by screening 200 samples across Africa from the *Anopheles gambiae* 1000 genomes (Ag1000g) consortium project. The use of five distinct reference genomes available from the literature revealed significant disparities in read mapping efficiency, highlighting the importance of a pangenomic approach in this system. Using the Ngousso reference as a backbone for building the panreference, we identified substantial SVs within the reference, with ~10% (23Mb) as dispensable. De novo assembled SVs further augmented the reference genome with 1.5M new variants, totalling 521.4Mb (223% larger than

Ngousso) across the 200 genomes. However, only 15% (234k SVs; 82.1Mb) of these SVs uniquely anchored into the reference, and 3% (46k SVs; 15.4Mb) with high confidence. Notably, our analysis delineated distinct pangenomic dynamics between species, and also between the autosomes and X chromosome, particularly within heterochromatic regions. In particular, low-recombining regions previously identified as genomic "islands of differentiation" between *An. gambiae* and *An. coluzzii* were enriched in diagnostic pangenomic variations, dominated by young transposable elements. Moreover, SVs captured the key axes of genetic ancestry variation along the genome previously identified with single nucleotide polymorphisms (SNPs), including well-known chromosomal inversions and islands of species differentiation, implying that SVs are impacted by the same evolutionary forces. This study offers critical insights into the genetic diversity and evolutionary trajectories of *An. gambiae* and *An. coluzzii*, highlighting SVs, particularly transposable elements, as pivotal drivers in their adaptive and speciation processes.



Population genomics and structural variation underlying local adaptation to elevational gradient in a bromeliad from the Brazilian Atlantic Forest

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POSTER SESSION 2 - **P02.254**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

While structural variation (SV) represents a major source of genetic diversity, its contribution to adaptation in non-model organisms remains poorly understood. Local adaptation emerges through complex interplay between selection and genomic architecture, where both single nucleotide polymorphisms and SVs may play crucial roles. The diploid bromeliad *Pitcairnia flammea* Lindl.—distributed across steep elevational gradients in the Brazilian Atlantic Rainforest (zero-2,200 m)—provides an ideal system to dissect these mechanisms. Our prior work has established its populations as a model of phenotypic divergence adapted to an elevational gradient, yet the genomic basis of these adaptive strategies remains unresolved. Following on our previous work demonstrating phenotypic and ecological divergence among elevationally structured populations, we now employ a pangenomic approach to: (1) characterize how SV shapes adaptive variation across environmental clines, and (2) unravel the genomic architecture underlying tropical plant adaptation. We performed high-coverage whole-genome resequencing (Illumina NovaSeq, ~70× coverage) of 81 individuals representing eight populations distributed along the full elevational gradient of *P. flammea*'s range. Population structure analyses identified three distinct genetic groups, distinguishing the highland and lowland populations, and isolating an unique midland population. Genome-wide scans (FST, π and Tajima's D indices) identified 336 genes showing signs of positive selection

enriched for lipid-related metabolic processes and antioxidant production pathways, consistent with adaptation to elevation-specific stressors. These molecular mechanisms align with adaptations observed in our previous study on this species, where membrane lipid fluidity response was critical for tolerating temperature extremes across elevations. Furthermore, Genome-wide SV profiling, by using MANTA pipeline, identified approximately three million redundant SVs across all individuals, including 9,017 tandem duplications, 38,206 insertions, 61,202 deletions and 74,073 translocations. Notably, 48.83 Gbp of the short-read sequences did not align with the reference genome and is suitable to detect presence-absence variation (PAV). Our results demonstrate that local adaptation in *P. flammea* is shaped by both SNPs and SVs, revealing a complex genomic architecture of elevational divergence. While their relative contributions to adaptive processes require further validation (e.g., through functional assays or reciprocal transplants), these findings highlight the understudied role of SVs in tropical montane adaptation. As the first study to characterize genome-wide structural variation in a neotropical bromeliad, our work provides critical insights into the mechanisms underpinning climate resilience in biodiversity hotspots—particularly in the Atlantic Forest, one of Earth's most threatened yet understudied ecosystems.



Quantifying structural variants in chromosomes using landmark-based disparity

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POSTER SESSION 2 - **P02.308**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Chromosomal architecture has played a key role in the evolution of biodiversity. Detecting structural variants (SVs) on chromosomes has informed the study of speciation, sex determination, adaptation, and some of the earliest divergences in the tree of life. Here we present a computationally non-intensive approach, based on geometric morphometrics, that uses conserved DNA sequences as landmarks to quantify structural disparities of focal chromosomes across multiple species, individuals, or cell types. Using two approaches, we show that this 'geno-metric' method can be applied at micro- and macroevolutionary scales to discover and diagnose SVs. Based on human X-linked genes and ultraconserved elements as landmarks, we provide empirical demonstrations with amniote sex chromosomes, the *Drosophila virilis* group, and placental mammal genomes. Landmark-based structural disparity

analysis effectively identifies chromosomal rearrangements and has parallels with traditional morphometrics regarding chromosome size, landmark orientation and landmark availability. Using simulations, we show that structural disparity inferred from ultraconserved elements is correlated with overall levels of chromosome evolution; an attribute which is consistent with observed disparity between and within mammalian orders. We found that the disparity patterns of SVs have significant phylogenetic signal, giving them broad importance for studying evolutionary biology. Structural disparity analyses are a valuable addition to the comparative genomic toolkit in that they offer an intuitive, rapid mechanism for detecting SVs associated with single copy genetic landmarks and the potential to reveal broader patterns of chromosome evolution related to expansions, contractions, rearrangements and phylogeny.



Self-organization on a tight leash? Identifying the mechanisms of phenotypic robustness using fluctuating asymmetry in cichlid fish color patterns

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POSTER SESSION 2 - **P02.336**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Phenotypic robustness—the capacity for organisms to buffer their development against internal and external perturbations—is an essential mediator of the interactions between genomes, phenotypes, and environments. In populations with low robustness, genetic and environmental perturbations produce greater variation in phenotypes. Despite its importance in development, we understand relatively little about how phenotypic robustness works, e.g. the degree to which it is heritable, or whether the same mechanisms control robustness across levels of biological organization.

Materials and methods: We used fluctuating asymmetry of cichlid fish color patterns as an indicator of developmental robustness. To generate fish that varied in fluctuating asymmetry, we crossed two closely related cichlid fishes with similar vertically barred color patterns, but which differ in their rates of asymmetry: Chindongo demasoni (>95% symmetrical in lab populations), and Chindongo saulosi (varies from symmetrical to asymmetrical). We tested the heritability of fluctuating asymmetry using artificial selection for increased or decreased asymmetry, crossing the most- or least-symmetrical F₂ fish with each other. We also measured rates of color pattern asymmetry in wild individuals from Lake Malawi to compare with our lab populations. To measure asymmetry phenotypes, we used both automated, quantitative (a custom semantic segmentation model) and manual meristic approaches (bar counts). Finally, to identify regions of the genome associated with asymmetry, we also used whole-genome resequencing for n = 344 F₂ hybrids

and long-read genomes of the parents to perform quantitative trait loci (QTL) analysis. **Results:** In general, asymmetrical parents in our crosses had more asymmetrical offspring. We found that fluctuating asymmetry had a moderate narrow-sense heritability ($h^2 \approx 0.3$, $p < 0.001$) when measured as a quantitative trait, a result consistent across our selection regimes and replicates. Asymmetry as measured using bar count (absolute difference in left minus right) was not significantly heritable, underscoring the importance of quantification in complex traits. However, we did find that bar number itself was highly heritable ($h^2 = 0.84$). We also identified a genetic basis of meristic bar variation and recovered a major effect locus for a phenotype of five bars (the C. demasoni pattern) vs. six or more (as in C saulosi). Interestingly, fish with the C. demasoni-like five-bar phenotype exhibited lower rates of asymmetry (30%) than fish with six or more bars (44%) suggesting interactions between factors influencing color pattern characteristics and symmetry.

Conclusions: Our results support fluctuating asymmetry as having a significant heritable component, and suggest a link between asymmetry and overall color pattern variation in development. Such a link could explain our observation that fish with the five-bar locus were also more likely to be symmetrical: a more robust patterning mechanism will often produce the same phenotype on both sides. Our findings therefore provide a compelling direction for investigating the interaction between phenotypic diversification, developmental robustness and heritability.



Sexually antagonism and the mating system determine the rate and distribution of recombination suppression across sex chromosomes

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POSTER SESSION 2 - P02.184, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The suppression of recombination between sex chromosomes is a widespread feature of genetic sex determination systems. Such recombination arrest is thought to trigger sex chromosome divergence and degeneration and has evolved multiple times in homomorphic ancestors, making it a striking case of convergent evolution.

Existing models for the evolution of recombination suppression fall into two main categories: those that rely on the prior segregation of sexually-antagonistic alleles and those that do not. Using mathematical modelling, we compare here the tempo of recombination evolution via the fixation of neutral recombination suppressors with those capturing a locus contributing to a sexually antagonistic trait. We do this for a trait locus with fixed effects and for a locus where allelic effects evolve. We show that sexual antagonism significantly accelerates the substitution of recombination modifiers

relative to genetic drift alone, though the strength of this effect depends on the nature of sexually antagonistic variation and on the mating system. Where allelic values at the trait locus evolve, sexually antagonistic selection leads to a pattern of male heterozygote advantage that results in especially rapid substitution by recombination modifiers. We also find that recombination suppression driven by sexual antagonism often arises on the homogametic chromosome, which is not the case when suppression evolves solely due to drift. This pattern is especially strong in XY systems where male reproductive success is highly variable. Our results highlight sexual antagonism as a key driver of recombination arrest, and suggest that reproductive ecology may play a previously underappreciated role in the evolution and stability of sex chromosome systems.



Squalomix: genomic exploration of shark and ray evolution

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POSTER SESSION 2 - P02.262, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The class Chondrichthyes is one of the most ancient evolutionary lineages of vertebrates understudied particularly at the molecular level. This group, which includes chimaeras, sharks, and rays, exhibits remarkable phenotypic diversity and occupies a wide range of ecological niches, primarily in marine environments. To investigate the molecular basis of their evolutionary diversification, the Squalomix consortium was established through collaborations with aquariums and marine stations across Japan. This initiative provides a wealth of DNA sequence data and practical technical frameworks (Nishimura et al. 2022, F1000Res 11:1077). Our initial analyses of shark species inhabiting Asia-Pacific waters (brownbanded bamboo shark, cloudy catshark, and whale shark) revealed reduced molecular evolutionary rates in chondrichthyan lineages (Hara et al. 2018, Nat. Ecol. Evol. 2:1761-1771). This finding suggests the potential for better preservation of ancestral genomic characteristics at early vertebrates or gnathostomes, in

chondrichthyan genomes. Further studies have uncovered unique evolutionary patterns in shark visual opsins, which may be linked with adaptation to deep-sea life (Yamaguchi et al. 2023, PNAS 120:e2220728120), as well as molecular signatures associated with the plasticity of reproductive modes in chondrichthyans ranging from lecithotrophy to matrotrophy (Ohishi et al. 2023, Genome Biol. Evol. 15:evad028). From a genome evolution perspective, chondrichthyans display substantial variation in genome size and karyotype structure, making them an invaluable model system for exploring the potential biological significance of their abundant genetic materials and chromosomal organization (reviewed in Kuraku 2021, Dev. Biol. 477:262-272). In this presentation, the activities of the Squalomix consortium will be summarized to highlight its potential to understand the unique evolutionary and ecological adaptations of chondrichthyans in a changing world.



The complex genomic landscape of inversion polymorphisms in the spruce bark beetle: genomic patterns, evolutionary forces, and implications for evolutionary inference

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POSTER SESSION 2 - **P02.380**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The diversity of life can manifest itself at many different levels, including the diversity of intraspecific variation in genomic architectures. Among these, polymorphic inversions are often found to underlie complex phenotypic polymorphisms and facilitate local adaptation in the face of gene flow. In addition, a growing body of research is providing evidence for genomes with dozens of large inversions segregating within species, raising questions about the extent of inversion landscape complexity, the evolutionary forces that maintain/influence such diversity, and our ability to account for this complexity in evolutionary inference. We have recently characterized a spruce bark beetle genome that contains 27 large polymorphic inversions covering ~30% of the genome. Inversions vary in size and degree of intra-inversion recombination, are highly polymorphic across the species range, and often overlap, forming a complex genomic architecture. Inversions are enriched in selection signatures and also harbor significantly fewer transposable

elements than the rest of the genome. They are enriched in odorant receptor genes, one of which has been confirmed to carry a functional olfactory polymorphism oriented towards different pheromone components. The existence of many inversions with intermediate frequencies suggests the role of balancing selection in their maintenance. So far, we have found no support for mechanisms such as overdominance and associative overdominance and are now investigating temporal changes in inversion haplotype frequencies over several generations. In addition, selection scans and demographic inference based on the inversion-rich genome of the species have shown that the presence of complex inversion polymorphisms can affect inference to varying degrees. Our results indicate that the species genome harbors one of the most complex inversion landscapes described to date, raising questions about the limits of complexity of intraspecific inversion variation and its influence on evolutionary inferences.



The genomic landscape of historical and ongoing gene flow in hybridising *Geum* (Rosaceae)

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POSTER SESSION 2 - **P02.384**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The genomic landscape of gene flow between hybridising species can change over evolutionary time, due to the establishment of new barrier loci or the breakdown of existing ones. However, relatively few studies have quantified the temporal dynamics of such barriers, which can be approximated by comparing gene flow landscapes between hybridising species in different geographical contexts. Specifically, allopatric samples reflect genomic signals of historical gene flow, while hybrid swarm samples mainly capture the outcomes of ongoing gene flow.

We sample two hybridising plant species, *Geum urbanum* and *G. rivale*, from 39 allopatric populations and three hybrid swarms in the UK. Using model-based demographic inference (gIMble) on whole genome resequencing data, we first quantify heterogeneity in effective migration rates along the genome and identify potential barrier regions. We then estimate genome-

wide hybrid indices using diem, which polarises genomic sites using an EM algorithm. The inferred site-specific ancestry is then visualised by chromosome painting.

We find asymmetrical gene flow between the focal *Geum* hybridising pair. The genomic landscape of gene flow is highly congruent between allopatric and sympatric populations, with more introgressed regions clustered toward chromosome ends. However, we inferred an additional 5.8% of the genome (75 Mb) acting as a barrier to gene flow in allopatric samples only.

This study presents a comparative perspective on how genomic landscapes of gene flow change over evolutionary timescales. It also highlights the value of recently developed population genomics approaches for characterising barrier regions, which are fundamental for understanding the maintenance of species boundaries.



The good, the bad or the lucky? How TE-driven hybrid dysgenesis interacts with a globally adaptive insecticide resistance locus

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POSTER SESSION 2 - **P02.365**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Understanding how populations respond to anthropogenic pressures provides key insights into the genetic basis of rapid adaptation. Transposable elements (TEs) are a major agent of rapid genomic evolution. Though common in most organisms, TEs can inflict major functional impacts on their hosts. Generally, the vast majority of functional TEs are expected to be deleterious, yet there are numerous examples of TEs providing adaptive benefits, often within remarkably rapid timeframes. The P-element - a form of class II transposon - represents an intriguing case of potentially conferring simultaneous adaptive and deleterious impacts in one of its host species, *Drosophila melanogaster*. While P-elements have only recently invaded the *D. melanogaster* genome, their role in hybrid dysgenesis (HD) is well-characterised. More recently, a P-element insertion into the Cyp6g1 locus has been demonstrated to confer significant levels of resistance to insecticides globally. However, alongside this adaptive benefit, the hybrid dysgenesis caused by this P-element may have implications for the ability of this beneficial resistance allele to spread through populations. Furthermore, other TE families (I elements; hobo elements) can also cause HD, but it remains unclear how these TEs may interact with each other in natural populations. While short-read sequencing projects have characterised selective sweeps surrounding the Cyp6g1 locus in

both North America and Europe, this approach has been insufficient to fully resolve this complex region. With a pan-genomics based approach, we are building a reference graph for the Cyp6g1 region spanning North America, Europe and Australia. Publicly available re-sequencing data across these continents enable us to identify the specific variants driving these selective sweep signals on each continent. We then characterise whether the Cyp6g1 BP allele (containing the P-element) is indeed confined to only Australian populations. Furthermore, the pan-genomics approach enables us to leverage both publicly available and novel genome assemblies to accurately assess TE load caused by P-elements, as well as I-elements and hobo elements across each continent, as this may indicate population-level signals of interaction between the HD-causing TEs. Alongside this analysis, we perform crosses between *D. melanogaster* populations across the Australian continent to assess the phenotypic impact of TE load in causing HD. This natural system enables us to not only understand and quantify the role that TE-derived HD plays in natural populations, potentially affecting the spread of resistance alleles, but can also serve as a useful proxy for further examining how the genetic background may facilitate or hinder the spread of a globally adaptive variant towards fixation.



The joint evolution of separate sexes and sexual dimorphism

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POSTER SESSION 2 - P02.385, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Dioecious plants are frequently sexually dimorphic. Such dimorphism, which reflects responses to selection acting in opposite directions for male and female components of fitness, is commonly thought to emerge after separate sexes evolved from hermaphroditism. But associations between allocation to male and female function and traits under sexual conflict may well also develop in hermaphroditic ancestors. Using mathematical modelling, I will first show that variation in sex allocation and a trait under sexual conflict inevitably generates an advantage to sexual specialisation, fuelling the simultaneous emergence of separate

sexes and sexual dimorphism. I will then consider how such association might materialise genetically, via recombination suppression or sex-dependent expression, and show that the genetic architecture of sex allocation and the conflict trait readily evolves to produce the association favoured by selection. Finally, I will discuss how pervasive ecological characteristics of flowering plants such as limited dispersal and self-fertilisation can offset the advantage of sexual specialisation generated by sexual conflict and thus maintain hermaphroditism.



Translocations and climate glacial refugia shaped the genetic structure of the largest Italian wild ungulate, the red deer *Cervus elaphus*

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POSTER SESSION 2 - **P02.383**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Many wild ungulates are prized as game species and are consequently influenced by human activities, including extensive translocations, the introduction of non-native species, hunting and active management. The red deer (*Cervus elaphus*) is one of the most iconic wild ungulates in Europe, playing a crucial ecological role in forest ecosystems. Until the tenth/eleventh centuries, the red deer was distributed over most of Europe, and it was still a common species in almost all the Italian mainland. During the late Middle Ages and the Renaissance, its distribution range decreased considerably, due to gradual deforestation and hunting pressure. Through the XX century, the species was instead subjected to several reintroductions across its past distribution, undergoing a spontaneous demographic and distributional expansion. Understanding the genetic structure of deer populations is essential for effective management and conservation strategies, especially in light of increasing habitat fragmentation and human-wildlife interactions. This study aims to investigate for the first time the genetic diversity, population structure, and potential barriers to gene flow among red deer populations across different regions of Italy.

Materials and methods: We collected samples of red deer (*C. elaphus*) from various localities, both protected and managing Italian areas: Lombardy (n=9), Trentino Alto Adige (n=14), Friuli Venezia Giulia (n=3), Piedmont (n=14), Liguria (n=2), Emilia Romagna (n=9), Tuscany (n=101), Abruzzo (n=5), Apulia (n=1), Campania (n=1), Calabria (n=2), Sicily (n=1). We sequenced a portion (950 bp) of the mitochondrial DNA control region (mt CR, D-loop) in 162 individuals.

Results: We described 42 haplotypes out of the 162 sequences with a haplotype (gene) diversity $H_d = 0.880$, a nucleotide diversity (per site) $\pi = 0.013$, a total number of mutations $\Theta = 70$ and a number of polymorphic sites $s = 68$. Haplotypes constitute three deeply distinct haplogroups corresponding to A, C, D lineages already described for the rest of Europe. Conventional F-Statistics detected highly significant values among lineages ($F_{ST}/\Phi_{ST} 0.3/0.4$ $p < 0.001$). Considering the entire Italian population, the AMOVA tests provide data for a strong genetic structure with an interpopulation variation of 28.78% and 71.22% within populations. Both phylogenetic and genealogical relationships supported the existence of different lineages coexisting in the same area.

Conclusions: The co-occurrence in the same area of haplotypes belonging to different lineages provides substantial evidence for how historical climate fluctuations, geographical barriers, and human activities have shaped the genetic landscape of red deer in Italy. The observed pattern is thought to mainly result from isolation in separate refugia in the Iberian Peninsula and the Balkans during the last glaciation. Consequently, these local phylogeographical patterns appear to reflect post-glacial recolonization processes influenced by the geographic positions of refugia and natural barriers to gene flow. Despite the red deer's significance as a game species and a long history of translocations across Europe, we didn't find individuals that did not align with the three main lineages—suggesting that most translocations have occurred over relatively short distances.



Uncovering the Genomic Architecture of Tooth Development Across a Dietary Radiation of Bats

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POSTER SESSION 2 - P02.255, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Being at the interface with the environment, tooth morphology has been shown to evolve rapidly in response to dietary and adaptive pressures. As a result, vertebrates, in particular mammals, have evolved a remarkable diversity of tooth shape in tandem with dietary diversification. Over the past decades, extensive studies in mice and a few other mammals have significantly advanced our understanding of the genes involved in the tooth gene regulatory network. Yet, our understanding of how differences in regulating gene expression in space and time contribute to tooth diversity is still limited. To study this question, we use the New World leaf-nosed bats (Phyllostomidae) as a model system. During their adaptive radiation, this group has colonized a wide range of ecological niches, including insectivory, carnivory, frugivory, nectarivory, and sanguivory. In parallel, they have evolved a striking diversity of tooth shapes, making them an ideal system for investigating how genomic architecture underlies adaptive morphological divergence. Thus, as part of a broader project on the evolution of bat tooth development, we present exploratory findings from a comparative genomic screen for gene regulatory elements involved in odontogenesis.

Leveraging a dataset from the Bat1K consortium, including 103 chromosome-level genome assemblies and reference-based whole-genome alignments, we performed a bioinformatic screen to identify conserved non-coding elements and

lineage-specific accelerated regions in clades with extreme dietary and dental phenotypes.

To link these regulatory candidates to the genes implicated in dental development, we integrated bulk RNA-seq data from several bat species sampled at key embryonic stages of craniofacial and tooth development. We then cross-referenced differentially expressed genes with nearby accelerated or conserved non-coding regions to identify putative cis-regulatory modules active during odontogenesis.

Our preliminary results reveal candidate lineage-specific regulatory signatures near genes involved in dental patterning, including both enhancers and repressors that may contribute to tooth gain, loss, or reshaping. Several of these regions may coincide with known chromatin-accessibility peaks in other mammalian models, making them promising candidates for further experimental validations.

Our integrative framework, combining taxon-rich comparative genomics with developmental transcriptomics, illuminates how variation in gene regulation can drive phenotypic diversification across rapidly radiating lineages. By uncovering regulatory mechanisms underlying tooth development in bats, we contribute to a broader understanding of how genetic architecture evolves in response to ecological pressures, specifically, the in situ dynamics of gene regulation during dental morphogenesis.



Understanding the costs and benefits of pleiotropy for adaptive evolution in an in silico model of serial organ evolution

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POSTER SESSION 2 - **P02.321**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Pleiotropy is often considered to act as a brake on evolution: a mutation that might have a positive effect in a given organ can be counter-selected if it is deleterious in another organ. Serial organs offer a particularly interesting model to study this hypothesis, because they share a common (pleiotropic) developmental program that is modulated by organ-specific (modular) identity genes. In theory, modular - rather than pleiotropic - mutations should therefore mainly be responsible for phenotypic variation in a given organ. However, experimental evidence from our lab has shown that pleiotropic developmental changes can support adaptive phenotypic change in one organ (upper molar), while they are compensated in another organ (lower molar), leaving its phenotype unchanged. This discrepancy suggests a previously overlooked decoupling between developmental and phenotypic pleiotropy, which we investigate in this work.

In order to understand more precisely this difference between developmental and phenotypic pleiotropy, we developed an in silico experimental evolution framework based on a paradigmatic example of serial organs: the rodent molar. The basis of this framework is ToothMaker, a tooth development model that incorporates regulatory networks and biophysical constraints to obtain 3D tooth shapes according to a set of input parameters. We use ToothMaker to simulate the development of the upper and lower tooth of artificial individuals. Then, we evaluate individual fitness based on tooth occlusion (the contact surface between the upper and lower tooth) to select reproducers, and create the next generation by generating random mutations in the genome of reproducers. Using this framework, we simulated the evolution of two populations defined by their contrasting genetic architectures: with and without

pleiotropy. In individuals with pleiotropy, lower and upper tooth input parameters are obtained by summing a shared gene parameter (representing shared molecular regulation of pleiotropic genes) and a tooth-specific gene parameter (representing tooth-specific molecular regulation of pleiotropic genes by identity genes). In individuals without pleiotropy, each tooth has its own separate set of genes, allowing for fully decoupled evolution of input parameters and hence of tooth shapes.

Using this model, we previously showed that populations with pleiotropy reached better fitness than populations without pleiotropy, and that many adaptive mutations lay in the pleiotropic part of the genome (Hagolani et al., bioRxiv 2025). However, we could not study the phenotypic effect of individual mutations. In this work, we have developed a new analysis that now allows us to track the fate of every mutation throughout the lineage of all individuals. We will use it to quantify both the fitness effect and *effective pleiotropy* of mutations in pleiotropic individuals, which we define as the relative amount of phenotypic change a mutation causes in the upper and lower tooth: this can vary because of tooth-specific regulation by identity genes, and because of epistatic interactions with constitutive parameters. By systematically evaluating epistasis between all pairs of fixed mutations in a given lineage, we will also be able to measure to what extent the underlying fitness landscape, due to epistatic interactions within a given genetic background, is itself under selection in pleiotropic populations. Overall, we expect this work to show that pleiotropy can in fact bring upon higher evolvability by allowing for developmental system drift, selectively neutral molecular and developmental changes that are not reflected in phenotypic traits, a mode of evolution which is not possible in modular systems.



A mosaic of modular variation at a single gene underpins convergent plumage coloration

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The reassembly of genomic variation from multiple origins is an important contributor to phenotypic diversification, yet insights into the evolutionary trajectories of this combinatorial process and their interplay with genetic architecture remain scarce. Here, we show how a modular, monogenic architecture shaped the evolution of convergent plumage coloration in a recently radiated species complex within the wheatears (genus *Oenanthe*). Among the four species studied, a black-or-white throat color polymorphism segregates within three species, while each species is fixed for either black or white mantle coloration, with mantle color phenotypes shared by non-sister species. Leveraging the power of multiple hybrid zones, we first uncover the genetic basis of these plumage traits. Genome-wide association studies reveal that both color traits are underpinned by variation at the same locus, which encodes the agouti signalling protein (ASIP). Yet, throat and mantle coloration are associated with different types of variation at this locus. The throat color polymorphism is largely explained by an interaction between a long terminal repeat (LTR) retrotransposon insertion and coding variation within ASIP, while mantle coloration is associated with non-coding variation found up to 50kb upstream of ASIP. An isoform-sequencing experiment supports the roles of both the throat and mantle associated genetic variation in the regulation of ASIP. Guided by this in-depth understanding of the genetic basis of coloration, we then investigated the evolutionary history of phenotypic convergence

within these species, inferring ancestral recombination graphs to estimate times to the most recent common ancestor (TMRCA) among different coloration haplotypes. We show that introgression of the LTR and protein-coding variation underpins the trans-specific throat color polymorphism, which stable isotopes suggest are associated with alternative foraging niches. Additionally, we establish that both the white mantle and white throat variants arose in the same species. Convergent white mantle coloration is observed between the species where the white variation was thought to arise and the species that experienced introgression of the white throat variation. Thus, co-introgression of the regulatory mantle variation along with the throat variants likely drove convergent phenotypic evolution. The coding throat variants may have also introgressed into a black-mantled species, where white-throated individuals are observed at low frequencies. White mantle variation appears to be selected against within this species, suggesting that successful introgression of white throat variants required recombination to dissociate the white mantle variants in this context. The limiting factor imposed by the physical linkage of the mantle and throat variants may help explain why the white throat polymorphism remains rare in this black-mantled species, despite ongoing gene flow with a species exhibiting a substantial frequency of white throats. Our results illustrate evolutionary trajectories from pre-existing variation realized within the constraints of a monogenic architecture.



Chromosomal inversions, polymorphic duplications and parallel patterns of differentiation in a marine bivalve, the European flat oyster (*Ostrea edulis*)

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Marine bivalves are known for their structurally complex genomes, and the European flat oyster (*Ostrea edulis*) is no exception. In our study we used whole genome sequencing to analyse 34 flat oysters throughout the species range: 28 at moderate coverage (~20X) and 5 at high coverage (~65-160X) to investigate patterns of variation in the genome. Using the high coverage individuals, we discovered that up to 1/3rd of SNPs have an allelic coverage ratio that deviated from the 50:50 expectation in heterozygotes. K-mer analysis revealed significant proportions of k-mer pairs distant by one nucleotide demonstrating triploid-like (~15%) and tetraploid-like (~5%) profiles, despite no recent whole genome duplication events occurring in Ostreidae. High coverage enabled a detailed analysis of the covariation between relative sequencing depth and allelic coverage ratio of SNPs, demonstrating that a high proportion are located in polymorphic duplications (~10% of variants). Polymorphic duplications affect non-coding and coding SNPs equally but their density surprisingly varies between metacentric and subtelomeric chromosomes. In addition, using the moderate coverage individuals, we confirmed the presence of three polymorphic chromosomal inversions, also corroborated by the comparison of 4 chromosome level genome assemblies. Previous work has suggested that this

species may have a parallel pattern of differentiation occurring, leading populations at the two extremes of the range, east of the Mediterranean Sea and in the North Sea, to become genetically similar in certain regions of the genome. Chromosomal inversions are believed to play a role in parallel evolution and indeed two of the inversions demonstrated a parallel signal. Interestingly, we also identified a parallel signature in small genomic regions that are not inverted. We further explored these 'islands of parallelism' throughout the genome using genome annotation, determining that islands occur in genic regions, and exploring the co-incidence of polymorphic duplications. Finally, to better understand the processes that shaped these parallel patterns, we employed demographic history analyses, revealing a signal of secondary contact, suggesting that chromosomal inversions as well as the non-inverted islands retain a shared evolutionary history that has been erased by secondary gene flow in the majority of the genome. Our findings indicate that while chromosomal inversions may play an important role in parallel evolution, other genomic regions are also involved in this system. Our results provide further evidence of the structural complexity of marine bivalve genomes but suggest structural variation is only partly responsible for the parallel evolution in *O. edulis*.



Colour genes and clock genes: Genomic drivers of local adaptation in the wood tiger moth

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MEETING ROOM 117, August 20, 2025, 10:30 AM - 12:30 PM

Colour polymorphisms in natural populations are valuable for studying the evolutionary and genomic mechanisms that maintain genetic diversity. In the aposematic wood tiger moth (*Arctia plantaginis*), males exhibit a striking white–yellow hindwing colour polymorphism controlled by a 100Kb insertion containing a single gene duplication. Morph frequencies vary across space and time, with some populations also remaining monomorphic. To investigate the genomic mechanisms underlying this variation, we used long-read haplotagging sequencing of 300 individuals collected across four countries (Finland, Estonia, Scotland, Georgia) and over a 13-year timeframe. Our population genomic analyses revealed strong geographic

structure and differing demographic histories between populations, and we see evidence of selective sweeps at the colour gene. Beyond the colour locus, we identified multiple genomic regions with high genetic diversity that correspond to circadian clock genes, such as *period*, *timeless*, and *clock*, suggesting adaptation to local photoperiodic environments. Morphological traits (such as wing size and melanisation) also showed geographic differentiation, with GWAS hits linked to pigmentation and energy metabolism genes. Together our findings start to uncover how variation has evolved in natural populations and how puzzling aposematic colour polymorphisms are maintained



Convergent evolution through recurrent rearrangements and regulatory rewiring in the primate amylase locus

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MEETING ROOM 117, August 19, 2025, 11:00 AM - 1:00 PM

Recurrent gene duplications have emerged as major drivers of evolutionary convergence. However, the precise mutational mechanisms through which these duplications occur, and how they acquire novel expression patterns, remain elusive. The amylase locus in primates provides an ideal model for investigating such convergence. This locus exhibits remarkable structural and regulatory plasticity, with salivary expression independently and likely convergently evolving multiple times through recurrent duplications. Using high-quality genome assemblies from 53 primate species and transcriptome data from multiple tissues in Old World monkeys, we reconstructed the evolutionary history of the amylase locus across diverse lineages. We identified an ancestral duplication of the amylase gene in the ancestor of Catarrhini, which laid the foundation for recurrent segmental duplications and structural instability at the locus. Our analyses further revealed lineage-specific duplications driven by non-allelic homologous recombination, including secondary duplications within macaques, which carry three amylase gene copies, and independent duplications in baboons, which harbor four copies. In Old World monkeys,

salivary and pancreatic expressions are clearly linked to independent duplication events, confirming convergence via alternative routes to tissue-specific expression. Notably, the distal-most amylase gene in both baboons and macaques consistently shows elevated salivary expression, suggesting that the progenitor of the extant human AMY1 and AMY2A genes originated from a dual-function ancestral gene, subsequently undergoing neofunctionalization to gain tissue specificity in the great ape lineage. To further explore the functional consequences of these duplications, we conducted molecular evolutionary analyses and detected strong evidence of episodic diversifying selection acting on one of the lineage-specific amylase duplicates in baboons, consistent with functional divergence. We also annotated transcription factor binding sites within the promoter regions of all amylase paralogs and identified lineage-specific motif gains associated with salivary expression. Our findings highlight the modular nature of structural and regulatory evolution at this locus and illustrate how convergent phenotypes, such as salivary amylase expression, can evolve through distinct molecular pathways.



Deciphering Chromosomal Inversions And Their Evolutionary Dance In A Wing Polymorphic Beetle

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MEETING ROOM 117, August 19, 2025, 11:00 AM - 1:00 PM

Evolutionary adaptation can rapidly occur in response to environmental changes, ranging from allele frequency shifts at a few loci to highly polygenic scenarios across multiple chromosomes. Studying patterns of adaptive genetic evolution involving multiple loci and traits under high levels of gene flow is particularly interesting because these cases allow us to identify mechanisms that promote adaptation in the absence of reproductive or geographic isolation. In these cases, structural variations such as inversions can play a key role by preserving linkage of beneficial alleles. *Pogonus chalceus*, a saltmarsh beetle, provides such a compelling case of rapid adaptation and parallel evolution on a microgeographical scale. These wing polymorphic beetles exhibit short- and long-winged ecotypes adapted to different hydrological regimes. Short-winged beetles inhabit regularly flooded tidal marshes, while long-winged beetles reside in ephemeral marshes that are periodically inundated for months at a time. Divergent behavior, in which long-winged beetles disperse and short-winged beetles remain submerged when encountering inundation, likely drives habitat preference and reduces effective gene flow between ecotypes. Here, we investigated the genomic patterns of

divergence between ecotypes to identify possible genetic mechanisms that maintain these distinct ecotypes despite their close proximity (10-20 meters) and ample opportunity for gene flow.

New genomes were assembled for both ecotypes using PacBio HiFi reads and Hi-C chromatin capture. These assemblies are significantly less fragmented than the previously published draft genome, with BUSCO scores of over 98%. Population genetic analyses identified up to six extended regions of high divergence, hinting at large-scale inversions that facilitate adaptation in multiple traits. These regions cover over 10% of the genome and were identified in six chromosomes, including the sex chromosome. The high repetitiveness of the genome, alongside the high heterozygosity in sampled individuals, may contribute to the fragmentation seen at potential inversion breakpoints. Further research is needed to identify the punctual breakpoints of these inversions. In addition, we characterized the genes found within inversions and identified associations between the multiple inversions with variation in wing size as well as behavioral differences. These results provide unique insights into how genetic architecture drives adaptation and speciation.



Detecting polygenic selection in time series data.

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MEETING ROOM 117, August 19, 2025, 2:00 PM - 5:00 PM

Many traits are controlled by a large number of loci. Selection on such polygenic traits requires the change of numerous alleles, mostly from standing genetic diversity, especially in rapidly changing environments. Detecting signals of polygenic selection and disentangling the impact of selection from genetic drift is challenging. Selection leads to allele frequency changes, which are expected to be proportional to the fitness effect of the allele. Here, we developed and employed a method to identify the signature of directional selection on polygenic traits using time series datasets. First, we identified loci across the genome, which constitutently change their allele frequency over time and compared them to a permuted distribution of allele frequency changes in the absence of selection. Then, we used the

allele frequency change covariance over time between significant loci, to identify polygenic selection in the population. We employed our method in a replicated selection experiment from a maize landrace population that has been selected for three generations based on plant growth performance traits. As selection was based on genomic breeding values, we could compare the detected signals to known effect sizes. We show how standing genetic variation in maize enables the rapid response to selection through allele frequency changes at thousands of loci. Our results demonstrate the power of time-series studies in inferring polygenic selection signals and the involved genetic architecture.



Exploring unique genetic diversity in Dutch cattle breeds using a pangenome graph

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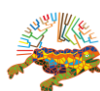
Background: Since the domestication of cattle, it has become one of the most prominent species on Earth with a population size of more than 150 billion. Due to different production goals, environmental conditions and cultural influences, a large variety of breeds has emerged. This has created a wealth of diversity, however, this diversity is hardly explored as most focus is on the larger production breeds. Meanwhile, traditional breeds are struggling in terms of population size, due to pressure on farming systems to become ever more efficient. In the Dutch cattle landscape the loss of population size in the local breeds has accelerated in recent years. the Netherlands is home to a variety of cattle breeds, but the only population marked as healthy has lost more than 25 % of its population in the last five years, while other breeds have shrunk by as much as 90 %. If the current trajectory continues, it is not unthinkable that some of these breeds could be lost in the near future. While it is difficult to reverse the processes that are leading to this loss of diversity, the least we can do is capture the genetics of these breeds in detail, and archive them.

Materials and Methods: We have used a combination of long- and short-reads to assemble de novo genomes of eight different Dutch cattle breeds. These were combined, together with the ARS-UCD2.0 cattle reference genome, into a reference-free pangenome. The resulting pangenome was evaluated using mapping rates comparisons of additional individuals with short-reads in contrast to the linear reference assembly, as well as visualisations of the pangenome structure. The pangenome was used to establish the amount of additional genomic

information present in the Dutch breeds. Individual variants unique to specific breeds were selected based on type of variant, size and location in the genome for further exploration.

Results: In this project pangenomes have shown their worth as a method to include more diversity within the species reference, not only providing a more accurate portrayal of the species, but also offering practical benefits such as better mapping rates for short-reads. Through this Dutch cattle pangenome we have established the genetic variance that exists within the Dutch cattle landscape, showcasing the unique variants that might be lost in the future. Furthermore, we provide an insight into the genetic diversity within these populations by looking at the amount of added variance per breed individual. Finally, by highlighting some unique variants found in these breeds, we showcase the use of new bioinformatic tools and methods for the in silico preservation of traditional, rare breeds.

Conclusions: This work has shown the value of archiving the genetics of traditional cattle breeds, by highlighting the genetic diversity that is found within local breeds, and showcasing the unique variants that are found within them. New bioinformatic methods, such as pangenomes, are well suited for the task of in silico preservation, as it allows for the inclusion of genetic diversity in the representation of the species. With these results we show that traditional breeds feature diversity not found in more common production breeds, and that their conservation is vital for preserving unique variants.



Gene copy number variations are untapped key players in adaptation along environmental gradient

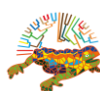
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MEETING ROOM 117, August 20, 2025, 10:30 AM - 12:30 PM

Gene copy number variations (gCNVs) are structural genomic alterations involving duplications or deletions that influence gene dosage and represent a significant source of genetic variation. While single nucleotide polymorphisms (SNPs) have constituted the primary focus of population genomics, recent studies indicate that gCNVs also play a pivotal role in adaptation. In this study, we investigate the role of gCNVs in local adaptation along environmental gradients in Norway spruce (*Picea abies*) and Siberian spruce (*P. obovata*), utilizing comprehensive genomic datasets derived from exome capture sequencing. A robust pipeline for the detection and quantification of gCNVs from short-read data has been developed, employing haploid samples for validation purposes. The results of our analysis demonstrate that gCNVs are pervasive, representing approximately 12% of targeted

genes. Notably, they are enriched in functional genes, particularly those associated with environmental stress, such as temperature tolerance, immune response, and metal ion regulation. Moreover, they followed the population structures similar to the ones identified through the analysis of SNPs, while also displaying distinctive adaptive signatures. Genotype-environment association (GEA) analyses and genome-wide association analysis (GWAS) demonstrate that gCNVs contribute to local adaptation and shape quantitative traits. By integrating SNPs and gCNVs, we demonstrate that gCNVs are capable of capturing patterns of selection that are ambiguous when using SNPs alone. Our study provides a solid framework to integrate gCNVs into population and quantitative genomics, and demonstrate their evolutionary importance.



Gene network topology drives the mutational landscape of gene expression

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Regulatory mutations, coding sequence alterations, and gene deletions and duplications are generally expected to have qualitatively different effects on fitness. Our objective is to ground this expectation within a theoretical framework using evolutionary simulations of gene regulatory networks (GRNs) controlling the expression of fitness-related genes. We examined the distribution of fitness effects as a function of the type of mutation and the topology of the gene network. Contrary to our expectation, the gene network topology had more influence on the effect of mutations than the type of mutation itself. In particular, the topology conditioned (i) the speed of adaptation, (ii) the distribution of fitness effects, and (iii) the degree of pleiotropy, acting as explanatory

factor for all mutation types. All mutations had the potential to participate in adaptation, although their propensity to generate beneficial variants differed according to the network topology. In scale-free networks, the most common topology for biological networks, coding mutations were more pleiotropic and over-represented in both beneficial and deleterious mutations, while regulatory mutations were more often neutral. However, this observation was not general, as this pattern was reversed in alternative networks. These results highlight the critical role of network topology in defining mutations' contributions to adaptation.



Genetics of repeated ecotype evolution in a Malawi cichlid

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Cichlids of the genus *Labeotropheus* are among the most widely distributed taxa at the rocky shores of Lake Malawi, likely due to an evolutionary innovation: their derived mouth morphology enables them to scrape and consume algae from rock surfaces that are inaccessible to other sympatric species. For over half a century, the genus was considered bitypic, consisting of a shallow-water species (*L. fuelleborni*) and a deep-water species (*L. trewavasae*), which primarily differ in body depth and male nuptial coloration where they co-occur. Based on whole genome sequences from more than 900 *Labeotropheus* individuals from diverse sites across Lake Malawi we show that the two nominal species are not monophyletic. Instead, a deep-adapted ecotype has repeatedly evolved from the more widely distributed shallow lineage,

each time coinciding with derived male nuptial coloration. Using our new whole genome sequencing dataset and chromosome-level assemblies we identified a putative inversion on chromosome 22 which is associated with ecotype assignment (shallow vs. deep) across geographically distant sampling locations. I will present these first genetic results and explore associations with ecological (habitat depth) and phenotypical (craniofacial morphology and pigmentation) traits in the context of adaptation and speciation. Due to its ecomorphological and genetic distinctiveness from other Mbuna species and the replicated differentiation pattern, the *Labeotropheus* system offers great potential to examine the genetic basis of speciation, the evolutionary process that creates biodiversity.



Genome reorganisation and expansion shape 3D genome architecture and define a distinct regulatory landscape in coleoid cephalopods

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Our understanding of how genomic changes translate into organismal novelties is often confounded by complex and multi-layered genome architecture, and the deep evolutionary time-scales over which genomic innovations can accumulate. Coleoid cephalopods (squid, octopus and cuttlefish) offer a unique system in which to study the impact of macroevolutionary genomic processes on phenotypic complexity. A large-scale chromosomal rearrangement in the coleoid ancestor (~450 MYA) resulted in an extensively modified karyotype and is hypothesized to underpin the evolution of coleoid innovations, including their large and elaborately structured nervous system, novel organs, and complex behaviours such as their dynamic camouflaging ability. Following this shared genomic shift, octopodiformes (octopuses and vampire squid) and decapodiformes (squid and cuttlefish) have also followed distinct evolutionary trajectories, shaped by lineage-specific chromosomal changes including fusions, translocations, repetitive element expansions, and tandem gene duplications. Yet, how these large-scale genomic

processes have impacted regulatory architecture remains poorly understood. To address this, we integrate Micro-C, RNA-seq, and ATAC-seq data across multiple coleoid species, developmental stages, and tissues, and examine syntenic genes, topological interactions and conserved non-coding elements (CNEs). We find that topological compartments tend to be conserved, yet exhibit divergent evolutionary paths depending on their ancestral context. In contrast, hundreds of putative regulatory chromatin loops are species-specific and often show stage- or tissue-specific activity with complex expression dynamics. Evolutionary analysis of such structures reveals a tendency of fusion and mixing in the octopus lineage, making it a more derived regulatory state. Furthermore, CRISPR knock-out of putative regulatory sequences within one such conserved region highlights its role in neural development. We propose that varying levels of relaxed evolutionary constraints on the coleoid 3D genome enables species-specific patterns of chromosomal rearrangement and expansion, facilitating the emergence of novel regulatory configurations underlying complex traits in the coleoids.



Genomic architecture and functional constraints associated with chromosomal fusions in natural populations

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Structural variants, such as chromosomal fusions, can compromise fertility, contributing to genetic barriers involved in speciation. Yet, the underlying evolutionary, molecular and cellular mechanisms remain largely unexplored. Here, we take advantage of wild populations of house mice (*Mus musculus domesticus*) from the Barcelona Robertsonian (Rb) System that naturally carry polymorphic chromosomal fusions to evaluate the impact of structural variants in the germline. To this aim, we combined transcriptomics and 3D genomics in the context of the meiotic dynamics. This allowed us to evaluate fertility costs and meiotic functional constraints associated with chromosomal fusions in natural populations. Overall, we detected increased heterologous chromosome interactions in

germ cells, especially affecting sex chromosomes. Such alterations were associated with changes in the 3D genome structure, transcriptomic profiles and recombination landscapes in mice carrying Rb fusions. Moreover, we detected differences in sperm head morphology among wild mice populations, suggesting that fertility costs are associated with the complexity of Rb fusions. Taking it all together, our results provide new insights into the effect of chromosomal fusions during spermatogenesis and how this is related to novel expression networks. We hypothesize that chromosomal fusions can rewire chromatin contacts, recombination rates and transcriptional landscapes, providing new grounds for evolutionary innovations.



Genomic Signatures of Coevolution and Climate in an Ant Social Parasitism System Reveal Links Between Signalling and Perception

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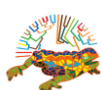
Coevolution between brood parasites and their hosts provides a powerful framework to investigate the genomic underpinnings of coadaptation. Although the phenotypic effects of social parasitism are well documented, the underlying genetic mechanisms remain largely unresolved. To address this gap, we studied the obligate slave-making ant *Temnothorax americanus* and its host *T. longispinosus* by integrating genome-wide association studies (GWAS), transcriptomic profiling, and analyses of chemical and behavioural traits. Our dataset comprises over 100 colonies from each of ten populations across the northeastern United States.

A first pool-seq GWAS on the host identified SNPs associated with parasite prevalence, particularly in genes involved in odorant perception and fatty acid synthesis. Host antennal gene expression also correlated with parasite prevalence, suggesting molecular adaptations for enemy recognition. Behavioural assays revealed population-level differences in host aggression, shaped by parasite prevalence and climate. Hosts from warmer, drier regions exhibited reduced aggression and prioritized brood retrieval, whereas parasites from the same regions showed elevated aggression. Cuticular hydrocarbon (CHC) profiles were shaped by different factors in each species: in hosts, climate was the primary predictor, while in parasites, CHC composition correlated with local parasite prevalence. These patterns indicate climate-dependent coevolutionary mosaics in chemical recognition traits.

To further investigate the genomic basis of adaptation, we conducted a 2nd GWAS study based on 120 resequenced individuals from both species, complemented by transcriptome

analyses of head and fat body tissues. Genes under selection in both species included those related to memory, behaviour, fatty acid metabolism, and circadian regulation. In the host, we identified many immune genes under selection through social parasitism, in particular peptidoglycan recognition genes, which are typically involved in antibacterial defences. Parasites showed selection on genes with transcriptional signatures associated with raiding behaviour. We also identified genetic variants associated with traits linked to recognition, aggression, and chemical communication. For example, in both species, we identified SNPs in genes underlying variation in cuticular hydrocarbon traits involved in fatty acid biosynthesis and chemosensory perception, consistent with a coevolution of signalling and recognition. Transcriptomic analyses revealed that the expression of many genes was shaped by both parasite prevalence and climate, with species-specific patterns: in hosts, constitutive expression of more genes was associated with parasite prevalence than with climate in contrast to the parasite where we found the reverse.

Integrating behavioural, chemical, genomic, and environmental data, we identify the genetic basis of key traits and show how climate and parasite pressure shape gene, their expression, chemical signalling, and behaviour. Our findings reveal distinct yet interacting selective pressures in hosts and parasites, driving geographically structured coevolutionary dynamics. This study underscores the combined impact of biotic and abiotic factors on coadaptation and the value of integrative, population-level approaches to understanding the evolution of social parasitism.



Highly modular genomic architecture underlies rapid adaptive radiation

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Some of the largest, youngest, and yet phenotypically most diverse adaptive radiations have evolved in the absence of geographic isolation. The dimensionality of phenotypic differentiation among their species is stunning but little is known about the genetic basis underpinning it. In Lake Victoria >500 cichlid fish species representing nearly as many different combinations of morphological and colouration phenotypes have evolved in <16,000 years. Analysing 120 of these species in a radiation-wide-association analysis, we show that the genetic basis of parallel evolved traits associated with ecological adaptation (craniofacial shape, dentition, body patterning) and sexual selection (nuptial colour motifs) is generally

polygenic and dispersed across the genome. Up to 50% of alleles associated with these traits are of ancient riverine origin. We find little pleiotropy among traits, which may reflect the ancient admixture history of variants and help bypass the ‘cost of complexity’ during diversification. Such trait modules act like Lego blocks, permitting many trait combinations to evolve finite elements. During speciation, long-distance modules of ecological and mating trait loci arise and are maintained via interchromosomal linkage disequilibrium. We propose that modular trait architectures emerging from admixture variation are key to combinatorial speciation happening often, making adaptive radiation ultrafast and phenotypically diverse.



How do Atlantic salmon respond to selective fishing under various genetic architectures of a life-history trait? An individual-based demo-genetic model as a virtual laboratory

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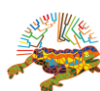
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The ability of species to respond to environmental change through adaptation of life history traits depends on several factors, including the rate of change, the genetic variation underlying fitness-related traits, but also the genetic architecture of these traits. In Atlantic salmon (*Salmo salar*), recent studies have shown that variation in age at maturity, a key life history trait, is strongly associated with a major effect locus. In addition, patterns of sex-dependent dominance have been shown to vary between populations. However, we lack understanding of the consequences of different genetic architectures underlying maturation on the evolution of life history traits and population dynamics of Atlantic salmon facing selective pressures such as environmental change and exploitation. We used an individual-based demo-genetic model, as a virtual laboratory to simulate the

life cycle of Atlantic salmon, together with the genetic basis of maturation and selective pressures. We compared alternative maturation genetic architecture scenarios (polygenic vs. major effect locus, dominance patterns) and assessed the demographic and evolutionary responses of populations to selective fishing. We observed a strong adaptive response of the population to selective fishing, with minor variations in evolutionary trajectories according to scenarios of locus effect sizes and dominance patterns. However, these variations do not influence the population at the demographic level. Our findings suggest that, at least within the framework of our eco-evolutionary model, the genetic architecture underlying life history traits might not influence population response to selective pressures in the manner previously anticipated.



One for all: the role of the FAR5 gene in a repeated polygenic alpine adaptation

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Evolutionary mechanisms of organismal adaptation are central to evolutionary biology. Due to its specific characteristics, the alpine environment provides an ideal model system for studying adaptation. Steep environmental changes along altitudinal gradients represent a challenge for survival that organisms must overcome. Moreover, its island-like character offers natural replicates, facilitating study of repeated adaptation to similar conditions.

This study focuses on repeated adaptation to the alpine environment in *Arabidopsis arenosa*, which has independently evolved an alpine ecotype multiple times. A previous study revealed a highly polygenic genomic architecture of this adaptation, involving several candidate adaptive genes. Despite the strongly polygenic nature of alpine adaptation, our work revolves around the role of a single gene. We aimed to identify a gene with a universal role in alpine adaptation—one that shows a strong signal of selection across all studied mountain ranges—and to assess the extent of its contribution.

One of the key strengths of this study lies in the broad range of disciplines it integrates. We present results from genomic analysis of natural variation, gene expression in yeast, a unique crossing design combined with transplanting plants back into their original alpine habitat, and metabolomic analysis in that environment. Using these approaches, we aimed to evaluate the role of a single strongly selected candidate adaptive allele within a complex polygenic framework.

We focused on FAR5, an enzyme involved in the biosynthesis of suberin—a hydrophobic protective barrier with diverse functions in plant life. We sought to uncover the function of the alpine allele of this gene. Natural variation in FAR5 reveals two distinct alleles, one prevalent in foothill and the other in alpine populations. Thanks to non-complete fixation of alleles, we were able to cross out alpine and foothill

homozygotes on both genomic backgrounds within the natural alpine and foothill populations. We then transplanted these plants into an alpine habitat and analyzed their metabolites in roots and wounded leaves using GC-MS. Additionally, we expressed both the alpine and foothill FAR5 alleles in yeast and examined the metabolic profiles of different genotypes under various temperatures.

Our field experiment showed that the alpine allele leads to increased production of shorter-chain fatty alcohol (C16:0-OH) compared to the foothill allele, which favors longer-chain alcohol (C18:0-OH). The effect of the alpine allele was additive, which we were able to assess using autotetraploid samples with varying degrees of heterozygosity. The greater the proportion of alpine alleles in a sample, the stronger the shift in substrate specificity toward production of the shorter fatty alcohol. The shift in substrate specificity was independently confirmed by transgenic expression in yeast, moreover this shift is influenced by temperature.

All in all, we show the impact of a candidate alpine allele on plant metabolomes in their native environment. Although its phenotypic effect appears modest—a slight shift in metabolite production—changes in substrate specificity may significantly affect suberin structure and function, with implications for water uptake, germination, and pathogen defense. Further investigation of its fitness effects and downstream impact of altered substrate specificity on suberin formation is needed to fully understand the role of this gene in alpine adaptation. This study demonstrates that even a gene with a small phenotypic effect can represent a sufficiently important component in a complex polygenic adaptation to an extreme environment to become a repeated target of natural selection.



Pleiotropy promotes preservation of genes when a phenotypic trait is lost: Evidence from knockdown of multifunctional fatty acid synthase genes

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Pleiotropy is a pervasive feature of genetic systems, as the ability of a single gene to affect multiple phenotypes has significant implications for trait co-evolution. However, the role of pleiotropic genes in shaping evolutionary trajectories when a phenotypic trait is no longer under selection has remained largely unexplored. Relaxed selection typically causes gene loss, but pleiotropy may prevent this because loss of pleiotropic genes would compromise other phenotypic functions.

Here we study gene pleiotropy under trait loss in the parasitoid wasp *Nasonia vitripennis*. Insect parasitoids have repeatedly lost the ability for adult lipid accumulation, yet the genetic machinery encoding fatty acid synthesis is still intact. We test the hypothesis that the preservation of fatty acid synthesis genes is due to pleiotropic effects on other functions than lipid accumulation, such as neurogenesis, pheromone production and cuticular hydrocarbon production (necessary for desiccation resistance and mate recognition).

We performed a series of RNAi knockdown experiments to understand pleiotropic effects of three paralogs of the key gene in the fatty acid synthesis pathway (*fas1*, *fas2*, *fas3*). First, we knocked down *fas1* and measured expression of eight other genes in the fatty acid synthesis gene network in the tissues where the other lipid functions are localized (head, male reproductive glands, and oenocytes, resp.). Tissue-specific differences were observed across the entire gene network, but specifically the expression of *fas2* in the oenocytes showed a strong increase, possibly compensating for the decreased *fas1* expression. Subsequently, we knocked down all three paralogs of *fas* and measured phenotypic functions. Total lipid content and starvation resistance were not affected, but desiccation resistance and mating behaviour were strongly decreased, consistent with the observed changes in *fas* expression patterns in the oenocytes. This study provides evidence for a conserving role of pleiotropy when phenotypic traits are lost, and reveals the intricate web of biological regulation in gene networks.



Speciation genomics in Bryophytes: unraveling the genetic architecture of reproductive isolation in haplo-diplontic plants.

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Background: A central goal in speciation research is to characterize the genetic architecture underlying reproductive isolation between species. Empirical patterns – such as reduced fitness in the F1 heterogametic sex or among F2 hybrids – suggest that many reproductive barriers may act through recessive and epistatic interactions in the early-generation hybrids. However, how this architecture translates into persistent barriers to interspecific gene flow over long evolutionary timescales remains an open question.

In this project, we aim to elucidate the genetic basis of reproductive isolation in bryophytes by leveraging their haplo-diplontic life cycle to investigate the roles of dominance and ploidy in the evolution of isolating barriers. Bryophytes life cycle is characterized by a dominant haploid, sexually reproducing phase (gametophyte) and a reduced diploid phase (sporophyte), both undergoing somatic development and having specialised structures. This unique biology offers a powerful framework to link genomic regions associated with reduced effective gene flow between species (i.e. reproductive barriers) to genes differentially expressed between life stages. In doing so, we can assess the contribution of recessive incompatibilities primarily expressed in hybrid gametophytes and underdominant effects specific to sporophytes.

Material and methods: We investigated five pairs of hybridizing bryophyte species spanning a broad phylogenetic range, representing Polytrichales, Dicranales, Hypnales, Pottiales and Marchantiales orders. For each species pairs, we generated population genomics data using Illumina whole-genome sequencing, sampling twenty-four individuals per species from sympatric natural populations as well as Illumina transcriptomic data from whole gametophytes and sporophytes of one species. Clustering analyses and genome scans provided, for the first time at a genomic scale, insights into the genetic structure of these bryophyte species.

Using model-based inference approaches, we identified genomic regions that locally restrict gene flow along the genome, while accounting for potential confounding factors. Finally, intersecting these results with differential expression analyses, we associated genomic regions of high or low effective gene flow with genes differentially expressed across the bryophyte life cycle.

Results: To date, only one species pair from the order Polytrichales – *Polytrichum commune* and *Polytrichum commune* var. *perigoniale* – has been analysed in detail. Although these taxa are recognized as varieties, our analyses revealed unexpectedly high levels of genome-wide differentiation (median $F_{st} = 0.83$) and sequence divergence (median $D_a = 0.04$), suggesting that this pair lies at a late stage of the speciation continuum. Despite that, we detected genomic heterogeneity with several regions showing signatures of interspecies introgression. Notably, one large ancestral chromosomal inversion exhibited low levels of differentiation and divergence. Demographic inferences supported recent gene flow concentrated in a few specific genomic regions, which we expect to be able to associate with genes predominantly expressed during the diploid sporophyte phase.

Conclusions: By extending this comparative genomic framework across multiple bryophyte species pairs, we aim to capture a broader spectrum of the speciation continuum and obtain a more comprehensive understanding of the genetic architecture underlying reproductive isolation. In particular, this approach will allow us to assess the role of recessive incompatibilities throughout the speciation process. Although largely overlooked in genomic studies, bryophytes offer a unique opportunity to contrast our findings with those from better-studied groups such as angiosperms, thereby enriching our general understanding of plant speciation.



Structural mutations set an equilibrium non-coding genome fraction

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The fraction of coding vs non-coding DNA is one of the main descriptors of genome architecture and is highly variable across the Tree of Life. Despite decades of debate, its determinants are still unknown. While some parts of the non-coding DNA arguably have a regulatory function, a large part does not seem to have a detectable impact on any phenotypic trait, the so-called Junk DNA. As such, the abundance of non-functional DNA in the vast majority of genomes throughout the Tree of Life challenges purely adaptationist explanations. Several non-adaptive theories, mainly based on population genetics, have been proposed to explain the presence of non-coding DNA and identify its determinants, emphasizing in particular the mutational hazard entailed by non-coding and non-functional DNA. In this view, the amount of non-coding DNA is driven by the level of genetic drift that determines the efficiency with which purifying selection can remove junk DNA.

Historically, the debate on the evolutionary determinants of non-coding sequences has therefore focused mainly on the question of selection, drift, and the balance between the two. But while these two forces are undoubtedly at the heart of evolution, evolution also requires a third force: variation. Indeed, one can state with absolute certainty that the genome architecture, and specifically the amount of non-coding sequences in a genome, can only vary through mutational events, including chromosomal rearrangements and small indels. Yet, the precise way these different types of mutations interact with the genome architecture has never been investigated in detail.

Starting from this very simple idea, we propose a mathematical model of the evolution of non-coding sequences. Assuming that the genome is partitioned into perfectly neutral and perfectly selected segments, we modeled various types of mutations susceptible to change the amount of non-coding sequences (typically chromosomal rearrangements and indels) and compute the probability for these events to be neutral (meaning they only affect the non-coding segments) or deleterious (meaning they affect one or several of the coding segments). The model shows how the non-coding fraction of the genome is shaped by two factors: an unavoidable bias in the neutrality of the different mutation types (adding base pairs is more likely to be neutral than removing some), and a strong robustness constraint imposed by the mere existence of chromosomal rearrangements. Indeed, rearrangements are more frequent and, on average, larger in larger genomes, imposing a strong second-order selection on genome size. We show that these two factors ensure the existence of an equilibrium non-coding fraction, which depends solely on the product of population size and mutation rate. Hence, by playing on these two factors -- and on their product -- the model is able to reproduce the full diversity of genomic architectures, from prokaryotes, for which it predicts a dense genome with a low, tightly constrained fraction of junk-DNA, to multicellular eukaryotes for which it predicts the accumulation of a substantial fraction of junk-DNA.

Reference: Luiselli, J., Banse, P., Mazet, O., Lartillot, N. and Beslon, G. (2025). Structural mutations set an equilibrium non-coding genome fraction. *bioRxiv*, 2025-02.



The evolution of sex-specific genetic architectures

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Sexual dimorphism represents one of the most striking displays of biodiversity, with profound differences in physiology, behavior, and life history recorded between the sexes across diverse taxa – all from a largely shared genome. While these phenotypic divergences are well-documented, the selective forces shaping their underlying genetic architectures remain poorly understood, particularly how sex-specific selection interacts with shared genomic constraints. We examine these dynamics with a rich single-cell RNA-sequencing dataset of the developing avian gonad of both males and females, covering species with varying degrees of sexual selection. First, we confirm that genes with

low pleiotropic constraint, including those exhibiting sex-biased expression, display elevated evolutionary rates. Secondly, we examine architectural properties of gene regulatory networks, and describe how the centrality of a gene and the connectivity of the network it lies in relates to its evolutionary trajectory. Finally, we describe the rates of turnover of sex-specific networks compared to those that are developmentally conserved. Together, these results highlight the complex interplay between gene regulation, evolutionary constraint, and sex-specific selection in shaping transcriptional diversity and provide novel insights into the regulatory underpinnings of sexual dimorphisms.



The evolutionary trajectory of seahorse inversions is shaped by multiple types of selection

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Recent empirical studies show that the genetic architecture underlying differentiation between ecotypes often involves chromosomal inversions. These structural rearrangements are thought to play a role in adaptation and speciation through the suppression of recombination between locally adapted alleles or genes underlying reproductive barriers. However, multiple processes influence the evolutionary trajectory of inversions which can be shaped by different types of selection, either simultaneously or at different stages in their evolution. Two inversions have been described in the long-snouted seahorse (*Hippocampus guttulatus*) and these show contrasting distribution patterns as well as other characteristics that suggest that they are maintained via different mechanisms. However, the type of selection acting on each inversion remains to be confirmed and their impacts on individual phenotype and fitness have yet to be discovered. Here, we analyse whole-genome and phenotypic data (n=96) from two seahorse populations that are each polymorphic for one inversion and fixed for the other. We used a haplotagging protocol to leverage linkage information to recover phase-resolved sequences at the genome-level. We polarised each of the inversions and evaluated their ages using an approach based on the bottleneck signal associated with the derived arrangement. To study the diversity of selective mechanisms acting on the inversions, we investigated to which extent they were associated with genetic load as well as with phenotypes such as sex, colouration and condition factor

(used as a proxy for physiological state). We measured mutation load using two different methods, one based on a comparative phylogenetic framework (GERP) and the other using the predicted functional effects of variants (SnpEff). We show that mutation load is higher in the inversions than in the collinear genome, and highest in regions not impacted by rare recombination events between arrangements, reflecting that purifying selection is less efficient when recombination is more scarce. The inversion showing the highest level of load also showed a heterotic effect with regards to condition, suggesting that heterozygous individuals had better fitness. Despite this effect that would favour polymorphism, other evidence shows that this inversion plays an important role in differentiating marine and lagoon seahorse ecotypes, indicating selection that favours divergence. The second inversion showed a significant association with colouration and with sex, with the consequence that it may be under balancing selection in order to maintain the sex ratio. Overall, our results demonstrate that seahorse inversions are impacted by an array of different selective mechanisms, and that the evolutionary trajectory of an inversion depends on the balance of these sometimes opposing forces. In spite of these differing mechanisms, the two inversions as well as potential interactions between them, appear to enhance reproductive isolation between seahorse ecotypes, suggesting that they together play an important role in adaptation and speciation in this species.



The genetic architecture of an adaptive phenotype conditions populations evolutionary response to climate change

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Populations can be locally adapted to environments that can vary across space and across time. Understanding the genetic architecture of phenotypic variation that enable natural populations to track temporal changes of the environment is crucial in the context of the ongoing climate change. One successful example of ongoing climate adaptation is that of chemotype diversity in common thyme (*Thymus vulgaris*). Thyme is locally abundant in Mediterranean landscapes and harbors a genetically determined chemotype polymorphism varying at a very local scale. Chemotypes can be grouped by their chemical composition into phenolic and non-phenolic ecotypes, which have been shown to be adapted to different local habitats. In recent years, ecotypes are shifting local frequency as they adaptively respond to ongoing climate change.

To understand the genomic architecture of this adaptive phenotype, we sequenced 1000 thyme individuals from over 70 sites spanning 9 independent gradients of altitude and environmental conditions across Southern France. We find weak population structure across populations, indicating abundant gene flow. We identify 3 genetic

loci associated with the ecotype, that together explain more than 90% of phenotypic variance at the ecotype level. All loci fall within genes that encode enzymes involved in monoterpene biosynthesis, and involve structural genomic variation such as copy number variants. We characterize the genetic architecture of the ecotypes and find that it involves epistatic interactions between these three loci.

We use on site temperature and soil measurements to characterize the local environment of some of the sampled transects. We then build genotype-environment association models to detect genetic variants involved in local adaptation. We find the majority of environment associated variants are also involved in determining the chemical ecotypes, confirming chemotype variation as the major phenotype mediating local climate adaptation in common thyme populations. Finally, we use population genetic forward simulations to explore if and why the inferred genetic architecture of thyme ecotypes provides an advantage over alternative genetic architectures for tracking environmental variation and climate change.



The Role of Sperm Competition and Genome Duplication in Cell Type Diversity in Fish Gonads

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Understanding how biodiversity arises is a key goal in evolutionary biology. However, only recently, has the idea of cell type diversity gained attention as recent studies have highlighted the importance of addressing cell types to understand the evolution of complex organs. But also, how the diversification of cell types parallels the dynamics observed in other units of biodiversity, such as species and populations. As this is an emerging field, few studies have contextualized the forces driving cell type evolution within the broader biology of species—largely due to the challenge of linking transcriptomic data to phenotype, and to the complexity of the evolutionary process and genomic histories shaping lineages.

Here, we aim to understand how gonadal cell type diversity evolved, by focusing on different species of teleost fishes—a group with a remarkable diversity of reproductive adaptations and a dynamic history of genome duplication. These characteristics allow us to investigate the interplay between cell type diversity, evolutionary forces, and genomic history. By selecting species with different levels of sperm competition, we hypothesize that cell type diversity was shaped by sexual selection. We are generating quantitative and spatially resolved transcriptomic atlases at cell resolution, and collecting phenotypic data at the cellular and tissue levels to correlate molecular findings with observable traits.

So far, we have generated data for the ovaries and testes of three species: zebrafish, medaka, and the broadnosed pipefish. We have begun the cellular characterization based on the expression profiles of cell type-specific transcription factors, following the evolutionary concept of cell type. This approach allowed us to identify cell diversity within germ and somatic cells of each gonadal tissue in each species. Furthermore, our comparative analyses within species (across sexes) have revealed shared and sex-specific cellular and molecular characteristics. For

instance, we find a correlation between germ and somatic cells across sexes, suggesting that this cellular division is driven by a core development program. Additionally, when comparing across species, we observe a similar correlation, indicating that the molecular program of these cell type clades may be conserved.

We have further identified specific transcriptomic dynamics of each sex. Notably, germ cells exhibit higher transcriptomic diversity in both tissues. However, testes show a greater number of differentially expressed genes compared to ovaries, something consistent across germ and somatic cells. These results suggest that testis cells are generally more transcriptomically diverse, and that the transcriptome of germ cells in both sexes tend to be more diverse than somatic cells. Across both tissues, we find that germ and somatic cells are characterized by the expression of single-copy genes, but somatic cells seem to have an enrichment of multiple-copy genes, in contrast with germ cells. Additionally, our preliminary analysis of gene specificity shows that genes expressed in testis germ cells exhibit higher specificity, compared to their somatic counterparts. These findings align with previous studies on the cellular evolution of testis in other vertebrates.

We are working on expanding our species sampling and correlating levels of sperm competition with cellular and organ-level phenotypes. This will provide deeper insight into how reproductive strategies and sexual selection have shaped the evolution of gonadal cell diversity in fish, and how genome duplication events have contributed to this process, offering a new perspective on the evolutionary forces shaping biodiversity at the cellular level.





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S48 - The maintenance of adaptive polymorphisms



A key protein of missing-self-induced innate immunity is polymorphic and driven by diversifying selection in a wild rodent

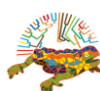
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POSTER SESSION 3 - **P03.229**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Host-parasite coevolution is known to cause the balancing selection driving polymorphism of host molecules involved in recognition of antigens and molecular patterns characteristic of pathogenic microorganisms. A way to induce immune response alternative to parasite recognition is the recognition of the absence of self-molecules. Although pathogens are known to evade destruction by such systems, how host self-identity markers evolve in response to such evasion is poorly understood. The missing-self system is involved in the so-called alternative complement pathway of vertebrates, in which the self-identity signal is conferred by complement factor H (CFH). CFH binds to polyanions specific to vertebrate cells, thus preventing their destruction. However, CFH is hijacked by

some pathogens, including the Lyme disease agent *Borrelia*. We investigated sequence variability of CFH in 13 populations of a wild rodent host of *Borrelia*, the bank vole. We found six variants that segregated both between and within populations. We detected a signal of positive selection at amino acid positions known to bind to *Borrelia* outer surface proteins, suggesting that CFH evolved in response to pressure from these pathogens. Furthermore, we found that the population structure of CFH is more pronounced than the genomic average approximated by RAD tags, indicating that CFH adapts to local pathogens. To our knowledge, this study is the first to demonstrate nonneutral CFH polymorphism in a wild vertebrate.



Associative overdominance can drive recombination suppression around loci under balancing selection

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POSTER SESSION 3 - **P03.220**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Genomic regions without recombination are more frequent than once thought in sexual organisms. Such non-recombining genomic regions are particularly frequent around loci with a permanently heterozygous allele such as sex-determining and mating-type loci. Although widely studied, the evolutionary explanations for the existence of such non-recombining regions are still debated. It has recently been suggested that the presence of recessive deleterious mutations in repulsion around permanently heterozygous loci (e.g. a mating-type locus) might set the stage for recombination suppression around such loci. While the fate of deleterious mutations has been the subject of several theoretical works, including around permanently heterozygous regions, the possibility of such maintenance of deleterious mutations has however been questioned and the conditions allowing the selection for recombination suppression remain unknown. Using

individual-based simulations, we demonstrated that permanently heterozygous locus (e.g. mating-type and sex-determining locus) or locus under balancing selection can facilitate the establishment of associative overdominance, i.e the formation of haplotypes of tightly linked recessive deleterious mutations in repulsion accumulating by drift and maintained by the selection against recombinant offspring, which in turn select for recombination suppression. These results shed new light on the mechanisms impacting genome evolution, notably in low recombining regions and around loci under balancing selection. This has especially implications for understanding the evolution of mating-type chromosomes, sex chromosomes, multi-allelic incompatibility systems and genomic regions under associative-overdominance.



Balancing, Linkage and Effects of Selection: a Deep Learning Approach

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POSTER SESSION 3 - P03.239, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The phenomenon of Balancing Selection (BS) observed across a multitude of organisms refers to the processes that sustain genetic variation over large numbers of generations in populations. After decades of debate about the role of BS, new life has been breathed into the topic of BS with advanced genomic techniques. The subtle nature of BS, and its complex interactions with factors such as LD have significant effects even over timescales longer than speciation events.

The implementation of polymorphism-aware phylogenetic models (PoMos) allows the inference of phylogenies and directional selection and more importantly BS with the recently developed PoMoBalance. We adopt a methodology that involves the training of neural network (NN) models, using convolutional NNs and feed-forward NNs. Features extracted from our data sets are fed into a machine learning framework. The NNs will thus be applied to data simulated with

SLiM in different scenarios combining LD and BS with corresponding ancestral recombination graphs (ARGs). Training and testing data are simulated for a great ape population scenario, incorporating the effect of dominance at intermediate allele frequencies that represent BS.

In the first instance, we rely on a large number of population genetics summary statistics (SS), including Tajima's D and site frequency spectra, among others. Additionally, we also rely on SS that summarise features of ancestral recombination graphs (ARGs) calculated from branch lengths, tree topology and lineage-through-timeplot. Here we present a comparison of the performance of the different sets of SS in the context of balancing selection and linkage disequilibrium. We also discuss an application to Thalassemias.



Characterising adaptive conflicts over sex-specific phenotypes in *Drosophila melanogaster*

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POSTER SESSION 3 - **P03.240**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Males and females of the same species often differ in their morphology, physiology and behaviour, reflecting the sexes' reproductive roles. But while sexual dimorphism is ubiquitous, recent work has highlighted that the divergence between the sexes is incomplete. There are ongoing adaptive conflicts between males and females of many species. Divergent selection on male and female phenotypes, in combination with genetic coupling between their traits, can lead to sexual antagonism (SA), where populations are polymorphic for genetic variants that are beneficial to one sex but detrimental to the other. There are several implications of SA; it plays an important role in the maintenance of fitness variation, shapes immune responses, and affects lifetime fitness and health. We lack understanding of the phenotypic traits that are at the centre of adaptive conflicts between the sexes and how they affect organismal fitness. In our lab, we have experimentally evolved *Drosophila melanogaster* under sex-limited selection for 27 generations, wherein we imposed competition among members of one sex while eliminating selection in the other sex. We characterised these populations that evolved under selection restricted to either males or females, to verify the opposing fitness effects of the variants they carry. We measured competitive adult fitness, wing shape morphology, and cuticular

hydrocarbon (CHC) profiles in males and females across the evolved populations and the founding population. For competitive adult fitness, we did not find males or females from either selection regime to significantly differ in their offspring production. Interestingly, the mean offspring production in males, irrespective of the selection regime, was higher than the founding population, while the females from both selection regimes produced about the same average offspring as the founder. Wing shape morphology exhibited overall differences between sexes, but also changed depending on the selection regime the sexes evolved in. Specifically, males from the populations where selection was limited to females exhibited a rounder wing shape, most often associated with a feminine wing shape. Females from the male-limited selection populations revealed a narrower, more elongated wing shape, which has been associated with a masculine wing shape. CHC profiles showed a more complex pattern, with sexes differing in their CHCs depending on the selection regime. Females significantly differed in their CHCs between the two selection regimes, however, they were not significantly different from the founding population males. Through this, we aim to provide nuanced insights into traits in *Drosophila melanogaster* that might exhibit antagonistic fitness effects.



Comparison of the the genetic structures of *Borrelia virulence* factor, the outer surface protein C, and the putatively neutral intergenic spacer

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POSTER SESSION 3 - **P03.230**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Borrelia afzelii, a major causative agent of Lyme borreliosis in Europe, is known for its high polymorphism of outer surface protein C (ospC) genes that help it evade the host immune system. The ospC protein is essential in the early stages of mammalian infection and is one of the first antigens recognised by the vertebrate immune system. Consequently, its polymorphism has been hypothesised to be due to balancing selection imposed by host immunity, which predicts that it should show a less pronounced genetic population structure compared to neutrally evolving genomic regions.

Here, we tested this prediction by contrasting among population differentiation of ospC genes with that of the rrs-rrl intergenic spacer region widely used as a phylogenetic marker characterized by a relatively high neutral rate of evolution.

Analysis of molecular variance (AMOVA) revealed that differentiation between populations explained only 1.84% of the total variance at the OspC, compared to 6.43% variance explained for rrs-rrl marker. The mean pairwise F_{ST} values for the intergenic region rrs-rrl (~ 0.066) region were significantly higher than those for ospC (~ 0), suggesting a stronger population structure for this neutral marker. The Mantel test revealed a moderate but significant correlation between genetic differences and geographic distance for the rrs-rrl region ($r = 0.3624$), while for ospC, this correlation ($r = 0.1958$) was not statistically significant.

These findings are consistent with the hypothesis that ospC is subject to balancing selection, leading to the maintenance of multiple allelic variants within populations across geographic range.



Deleterious hitchhiking under balancing selection as a potential fitness cost of balancing selection.

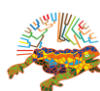
Stetsenko R¹, Hartfield M

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POSTER SESSION 3 - **P03.231**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Balancing selection maintains polymorphism among genetic variants and can originate from a diversity of biological mechanisms. Because balancing selection generates an increase in the frequency of heterozygotes, segregating deleterious mutations in closely linked regions can accumulate as they are masked by selection if they are partially recessive, creating a “sheltered load”. Previous theoretical works found that a sheltered load can be maintained under tight linkage with a site under balancing selection, mainly focusing on self-incompatibility in Angiosperms or mating-type loci in fungi. Moreover, empirical evidence for a sheltered load was found linked to the self-incompatibility locus in several Angiosperms and around the MHC region in humans. For relatively recent balanced polymorphisms, a substantial part of the deleterious load could be caused by deleterious mutations increasing in frequency along a new balanced allele. This deleterious hitchhiking could be increased in small and/or highly inbred populations because of the effect of drift and/or lower effective recombination. In particular, numerous genomic regions displaying

signatures of balancing selection have been described in self-fertilising species, despite very low genome-wide diversity caused by extreme inbreeding (such as in *Caenorhabditis elegans*). It is not yet clear, however, what could be the effect of different types balancing selection on linked deleterious mutations during the establishment of a balanced polymorphism since no general model exists in this case. We focus on the fate of deleterious mutations partially linked to a new variant under balancing selection when it spreads to reach its steady-state frequency. By using a semi-deterministic model, we derive approximations for the probability of partial hitchhiking and the sojourn time of deleterious mutations in a population of sexual haploids and compare them to results from individual-based simulations. We also explore the effect of inbreeding and its interaction with the type of balancing selection considered by subsequently adding selfing to the model. These results provide insight into how linked deleterious mutations can affect polymorphisms that are maintained by balancing selection.



Different Modes of Balancing Selection Leave Highly Similar Signatures in Time-Series Genetic Data

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POSTER SESSION 3 - P03.232, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Balancing selection is an important evolutionary force maintaining genetic diversity in natural populations. Emerging evidence suggests that there may be a larger number of loci than previously hypothesised under long-term balancing selection in human populations. Evolutionary mechanisms leading to balancing selection include heterozygote advantage, negative frequency-dependent selection, spatially and temporarily fluctuating selection and antagonistic selection. However, it is often not possible to pinpoint specific evolutionary mechanisms behind balancing selection signatures by using present-day genomic data. A promising approach to circumvent this problem may be to use time-series genetic data as the signatures of different balancing selection mechanisms left on genetic data may differ when looking through time. In this respect, ancient DNA data enables direct observation of allele frequency changes over time, allowing evaluation of theoretical models of balancing selection. In order to test whether ancient DNA data provides additional source of information, we conducted forward-time genetic simulations using SLIM under the

models of heterozygote advantage and negative frequency-dependent selection as well as neutral evolution. We incorporated population genetic parameters estimated for modern human populations in simulations and included temporal sampling at 1,000-year intervals for a total of 12,000 years coinciding the Holocene period. We then estimated 33 population genetics summary statistics including autocorrelations of these statistics through time on simulated data and used various machine learning tools to differentiate different modes of balancing selection. Results of support vector machines and random forest analyses indicate that both mechanisms of balancing selection can be distinguished from neutral evolution with high accuracy even with only present-day data. Meanwhile, it was challenging to discriminate between balancing selection modes when using present-day data as well as when utilizing time-series data. This indicates that these two modes of balancing selection leave highly similar genetic signatures through time and further investigations are required to develop methods to being able to distinguish them.



Differing adaptations in regulation of conjunctival immune response to a virulent pathogen across house finch populations

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POSTER SESSION 3 - P03.228, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Maintenance of adaptive intraspecific genetic variation is one of the key mechanisms driving host–pathogen co-evolution. Here, we present results of our gene expression analysis revealing regulatory adaptations in house finch (*Haemorrhous mexicanus*) inflammatory response to a bacterial pathogen *Mycoplasma gallisepticum* (MG). In North America, MG underwent a host shift from poultry to house finches in the early 1990s, leading to widespread mortality in the novel host and causing severe selective pressure across the populations affected. Previous research showed that long-term co-evolutionary interaction selected for tolerance to the pathogen in the house finches. We analysed conjunctival gene expression profiles 3 days after the host inoculation with MG in four house finch populations varying in their co-evolutionary histories with the pathogen (ranging from long-term exposure in Virginia and Iowa to limited or no contact in Arizona and Hawaii). Initial transcriptomics suggested variation in IL17 and IL12/IL23 pathways. Therefore, we compared the gene expression patterns in key cytokines regulating inflammation (IL1B,

IL4, IL10, IL12, and IL17) with those of BCL10, an NFkB signalling modulator. The cytokine expression profiles were significantly changed during the infection with MG. However, this shift was not consistent across the populations. In contrast to others, the birds from Virginia showed stronger down-regulation of IL4, IL12 and IL17 and diminished up-regulation of IL10. By comparison, the birds from Iowa showed greater up-regulation of IL1B and decreased BCL10 expression. Our results evidence distinct genetic signatures of the population adaptations to the pathogen. During the initial phase of immune response to MG, the more tolerant house finch populations select different response strategies: while in Virginia the birds generally attenuate proinflammatory responses, in Iowa they activate a strong IL1B-driven response that is dampened at the level of the signal regulator BCL10. Our findings illuminate the spatially varying selection in the avian host marked by differentially optimized immunological pathways undergoing regulatory adaptations during evolution of tolerance to a novel pathogen.



Gene conversion as a key contributor to adaptive polymorphism at the avian MHC

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POSTER SESSION 3 - **P03.226**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: The genes of the Major Histocompatibility Complex (MHC) are essential for vertebrate adaptive (acquired) immunity, as they encode pathogen-recognition molecules, which bind foreign antigens and present them to T cells. It is well established that high MHC class I and class II diversity increases the potential of an organism to recognize and neutralize a broader spectrum of intra- and extra-cellular pathogens, respectively. Hence, remarkable adaptive MHC polymorphism is often maintained in natural vertebrate populations via different forms of balancing selection. While this polymorphism is de novo generated by point mutations, new haplotypes may also arise by re-shuffling of existing variation through intra- and inter-locus gene conversion. However, the overall contribution of gene conversion to adaptive MHC polymorphism remains largely unknown.

Material and methods: In this study I used publicly available genetic resources to investigate a broad scale macroevolutionary patterns in gene conversion processes at the avian MHC. For this purpose, I retrieved and aligned ca. 9000 MHC class I and II sequences from nearly 200 bird species.

Results: I identified gene conversion as a universal and ubiquitous mechanism contributing to MHC polymorphism in birds. Over 80% of species showed footprints of gene conversion at either MHC class I or class II. In total, 25% of all analysed MHC allelic variants were found to have arisen through gene conversion. Although the processes of gene conversion were stronger at MHC class II than class I, inter-specific variation in gene conversion at both MHC classes arose through similar macroevolutionary trajectories. Finally, gene conversion at the MHC was primarily driven by gene copy number variation (consistent with significant contribution of inter-locus gene conversion) and species life history (high fecundity and migration promoted gene conversion).

Conclusions: The results indicate that the role of gene conversion in the maintenance of functional MHC polymorphism in natural bird populations may have been underestimated. The study shows that gene conversion should be considered a key contributor to avian MHC diversity.



Genetic basis and selection maintaining wing polymorphism in the evening brown butterfly, *Melanitis leda*

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POSTER SESSION 3 - **P03.225**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Colour pattern polymorphism, where multiple genetically-determined morphs coexist within a population, poses key questions on how such genetic and phenotypic diversity is generated and maintained. Although polymorphisms are known to contribute to adaptation and speciation, the underlying genetic mechanisms and the selective forces maintaining them remain poorly understood. The globally widespread cryptic Evening Brown butterfly, *Melanitis leda*, exhibits a striking wing colour polymorphism during the dry season, with individuals showing distinct variation in colours, bandings, and spotting patterns. Yet, the genetic basis and mechanisms maintaining such polymorphism within a population remain unknown.

Interestingly, this wing polymorphism is seen in all *M. leda* populations across the world, and the wide distribution of the species suggests that it is highly adaptable. Previous breeding experiments suggest that the polymorphism is controlled by a simple genetic architecture (Ruiter & Brakefield 1994), potentially maintained by balancing selection through negative frequency-dependent selection driven by predator search image formation.

This study adopts an integrative approach to investigate the genetics, development and evolutionary maintenance of wing pattern polymorphism. By combining phenotype quantification, genome-wide association mapping, time-series selection analysis, and developmental gene expression studies, we aim to disentangle both the origins and persistence of wing colour variation.

First, to investigate the genetic basis of the wing colour polymorphism in *M. leda*, we quantified dry-season morphs using phenotypic principal component analysis (PCA), distinguishing individuals based on wing colouration, banding, and spotting intensity. Using a full-sibling design from Indian populations, we estimated

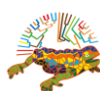
heritability for these traits. Preliminary results revealed high heritability, indicating strong genetic control and potential responsiveness to natural selection.

Second, to determine whether the genetic basis of wing colouration polymorphism remains consistent across populations, we will conduct a genome-wide association study (GWAS) using historical specimens of *M. leda* collected between 1987–1998 in Zomba, Malawi (van Bergen et al., 2016). We will combine morph classification from images with genomic sequencing data to identify loci associated with polymorphism. These loci will then be compared across additional populations from India, Ghana, and South Africa to assess whether variation arose from a shared ancestral origin or through repeated local adaptation.

Third, to identify selective pressures maintaining variation within the population, we will analyse the 12-year Zomba time-series dataset. By tracking morph frequency changes over time and linking them to environmental variables, we aim to assess whether balancing selection or fluctuating environmental pressures maintain the observed polymorphism.

Fourth, to explore the developmental genetic mechanisms underlying wing pattern variation, we will use Hybridisation Chain Reaction (HCR) to visualise the spatial and temporal expression of major candidate genes (*optix*, *spalt*, *cortex*) during late larval and early pupal stages. We conducted targeted dissections aligned with known developmental windows for wing pattern determination, including early and late 5th instar and 30% pupal stages.

By combining these approaches, this study aims to uncover the genetic and ecological mechanisms maintaining wing colour polymorphism in *M. leda*, contributing to a broader understanding of how phenotypic variation evolves and persists in natural populations.



Genomic Signatures of Sex-Specific Selection in the seed beetle *Callosobruchus maculatus*

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POSTER SESSION 3 - **P03.236**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Sexual selection is theorized to play a pivotal role in shaping genomic variation and architecture, either by purging deleterious alleles or by maintaining genetic diversity through balancing selection across the sexes. Despite its theoretical importance, empirical tests of this hypothesis remain limited. Here, we conducted experimental evolution spanning 90 generations in seed beetles maintained under three alternative mating regimes: polygamy (intense sexual selection), monogamy (eliminating sexual selection), and male-limited selection (restricting selection to males). By

comparing these regimes, we investigated how sexual selection interacts with natural selection to shape genomic variation, focusing on estimating the relative importance of purging of deleterious alleles and the maintenance of genetic variation through sexually antagonistic selection. By integrating transcriptomic and genomic analyses, we provide a comprehensive view of how mating systems influence genomic variation and pinpoint genetic mechanisms underpinning sex-specific adaptation and conflict.



Harnessing Simulations to Identify Balancing Selection in Evolve-and-Resequencing Experiments

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POSTER SESSION 3 - P03.237, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Natural selection is one of the key processes that governs how genetic variants become fixed, lost, or remain segregating within populations and species over time. Among other inferences, identifying alleles targeted by balancing selection is crucial to provide insights into evolutionary mechanisms underlying the maintenance of common variants. With the emergence of large sequencing datasets and the need for ever complex models, deep learning algorithms, trained by simulations, have shown great potential to detect cryptic signals of selection. Despite this, at the moment, only few studies employed deep learning methods to detect and characterise the genomic footprint of balancing selection, largely due to the complexity of producing high-fidelity simulations.

To address this gap, we sought to replicate a previously performed evolve-and-resequence experiment on sexual antagonism in *Drosophila melanogaster*. We implemented a novel, efficient, and flexible

pipeline to simulate various nuances of the experiment including variations in population sizes, distinct replicates, linked selection, temporal sampling, and uncertainty of sequencing data. Input data derived from the *Drosophila* Genetic Reference Panel were seamlessly incorporated into the protocol.

Using this framework, we demonstrated that generated synthetic data closely replicate allele frequency dynamics observed in the empirical experiment. Notably, we were able to highlight a region of interest with a pattern compatible with balancing selection. We further postulate and propose how such deep learning synthetic training data allows analysis in a model-agnostic way, allowing us to distinguish between different forms of balancing selection, a task that is still considered unachievable under most scenarios. We conclude by highlighting further technological advancement in devising deep learning algorithms for time-series genomic data.



Intramolecular trade-offs promote the maintenance of polymorphism in plant-pathogen coevolution

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POSTER SESSION 3 - P03.224, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Host-pathogen coevolutionary dynamics can lead to the diversification of genes involved in interactions between hosts and pathogens. In plants, pathogen recognition is often conferred indirectly via so-called 'guardee' molecules, which are targeted and modified by pathogen effectors to interrupt e.g. defence signalling. This modification is sensed by an interacting R protein, which adopts the role of a 'guard' and activates a specific defence response. It has been shown that the effector-guardee-guard interaction can be multi-dimensional. Many pathogen effectors can modify more than one target molecule in the host, many target molecules can be modified by several, often unrelated effectors and there are examples for gardees, which are guarded by more than one R protein. It has been proposed that, because of their pleiotropic role, there are potential evolutionary conflicts occurring at guarded target molecules. We assessed the evolutionary history of the guardee RCR3 in wild tomato species and revealed elevated levels of intraspecific diversity as well as

interspecific diversification and a signature of balancing selection. Investigating the functional characteristics of 54 natural RCR3 variants through in vitro and in planta assays, we detected differences in the interaction of the guardee with fungal and oomycete effectors, as well as substantial variation in the strength of the defence response. Our analyses pinpoint amino acid polymorphisms with key functional consequences for the coevolution between the guardee (RCR3) and its guard (Cf-2) and identify the coevolving sites between RCR3 and the interacting pathogen effectors. We conclude that in addition to coevolution at the 'guardee-effector' interface for pathogen recognition, natural selection acts on the 'guard-guardee' interface. Guardee evolution may thus be governed by intramolecular epistasis caused by trade-offs between improved defence activation in the presence and prevention of auto-immune responses in the absence of the corresponding pathogens.



Maintenance of Social and Wing Polymorphism in the ant *Myrmecina graminicola* through the selfish transmission of a haplotype

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POSTER SESSION 3 - **P03.234**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Supergenes lock specific combinations of genes by partially suppressing recombination and play an important role in the evolution of complex phenotypes. Due to the reduced recombination in the derived haplotype, it tends to accumulate selfish elements that promote its transmission. On the other hand, the derived haplotype also tends to accumulate deleterious recessive mutations (higher genetic load). These two opposing forces bias the haplotype transmission and play an important role in maintaining the polymorphism at the supergene.

In an increasing number of ant species, supergenes are known to determine the colony's social organization. In *Solenopsis invicta* and *Formica selysi*, whether a colony is monogynous or polygynous (i.e., one or several queens per colony respectively) is determined by a supergene. In both species, Mendelian transmission of these haplotypes is distorted. In *S. invicta*, adult queens lacking the derived haplotype (polygyne form) are killed by workers in polygynous colonies whereas in *F. selysi* the offspring of polygynous queens that do not carry this derived haplotype fail to hatch.

We tested for deviations from Mendelian ratios in the ant *Myrmecina graminicola*, where a social and a wing polymorphism are genetically determined by a supergene with three haplotypes: Mo, Ma, and Pa. MoMo queens are winged and establish monogynous colonies; MaMa and MaMo queens are wingless and also monogynous;

MaPa and MoPa queens are wingless and form polygynous colonies. PaPa individuals have not yet been found among queens but do exist among workers. Apterism is determined by a ~110kb insertion specific to Pa and Ma, but the molecular mechanisms hampering wing development are yet unknown. We will focus here on the distortion of M and P haplotypes independently of the presence of the insertion. In polygynous colonies, all the queens being heterozygous (MP), 50% of the diploid progeny should be heterozygous at the social supergene under Mendelian inheritance whatever the father. We tested whether there is a deviation from the expected frequency in workers at different developmental stages.

Using PCR-RFLP, we first genotyped 375 workers from 20 polygynous colonies at the social supergene. We detected a higher-than-expected proportion (65%) of heterozygotes (MP). From the same population, we are currently genotyping larvae (N = 210) and adult workers (N = 210) from 7 colonies. Because larvae could be haploid males and mistaken for homozygous diploids, we are developing microsatellite markers to assess the ploidy level of the larvae.

Regardless of the mechanisms, our preliminary results show an excess of MP workers supporting the occurrence of a distortion bias in favor of the derived haplotype. Whether this bias is also found in sexuals remains to be tested.



Molecular evolution in Bryophytes: How ploidy drives patterns and efficacy of natural selection.

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POSTER SESSION 3 - **P03.227**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The central theme of evolutionary biology is understanding the origin and fate of genetic variation. New mutations, which contribute to this variation, can follow different trajectories depending on their fitness effect. Mutations with largely beneficial or deleterious fitness effects may be fixed or lost by directional selection, while those with context-dependent fitness effects can be maintained by balancing selection. Most studies examine how differences in selection pressures between populations affect the fate of mutations. However, fewer explore how shifts in selection pressures over the course of an individual's lifetime influence mutation evolutionary dynamics. Complex life cycles provide a compelling scenario where selection regimes vary between different ploidy stages and interact with the dominance effects of mutations to determine evolutionary outcomes. Bryophytes represent such a promising model system as their haplo-diplontic life cycle alternates between two multicellular stages — a dominant haploid gametophyte, which undergoes sexual reproduction, and a minor diploid sporophyte, specialized in spore dispersal.

To investigate how ploidy and dominance affects natural selection in Bryophytes, we generated RNASeq data from both the haploid gametophyte and diploid sporophyte across five moss species scattered in different orders in the phylogeny. Here, we present results for one of these species, *Polytrichum commune* (order: Polytrichales). By assembling a high-quality de-novo transcriptome, we were able to assess differential gene expression using multiple dedicated tools. On average, ~18% of genes showed biased expression between the two life phases (fold change ≥ 2). Specifically, 11% of genes were biased toward the gametophyte and 7% toward the sporophyte. The remaining genes which form a majority, show unbiased expression across the life cycle.

We hypothesize that these gene categories, namely gametophytic-biased, sporophytic-biased and unbiased, are under different selection regimes. Genes with stage-biased expression may experience antagonistic selection, positive directional selection, or differential compensation of deleterious mutations between stages. Such dynamics could drive genetic divergence between the haploid and diploid stage, bringing each stage closer to its fitness optimum. Because the interaction between ploidy and dominance influences the efficacy of selection — with recessive mutations being masked in heterozygous diploids but fully expressed in haploids —, we expect mutations to have different evolutionary outcomes in the two life stages. Specifically, in the haploid stage, both purifying and positive selection are expected to act more effectively, facilitating the removal of deleterious mutations and the fixation of beneficial ones. On the other hand, synergistic selection — where both life stages share similar fitness optima — should result in sequence conservation of unbiased genes — which are likely involved in housekeeping functions. Meanwhile, unresolved evolutionary conflicts between life stages may be reflected in unbiased genes under balancing selection, maintaining ancient polymorphisms over time.

To test these predictions, we will couple population transcriptomic data from our various target moss species with genomic data from their respective outgroup species. This approach will enable us to ultimately obtain polymorphism and divergence estimates. Additionally, haplotype information will allow us to reconstruct ancestral recombination graphs for candidate genes, and date their age. By analyzing molecular diversity patterns across gene classes, we aim to deepen our understanding of the distinct selective forces acting on each, and more broadly, gain insights into the efficacy of natural selection in complex life cycles.



Survival, sex, and insecticides: decoding the mechanisms behind resistance polymorphism in *Drosophila melanogaster*

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POSTER SESSION 3 - **P03.223**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Upregulation of the cytochrome P450 gene Cyp6g1 in *Drosophila melanogaster* confers resistance to naturally occurring xenobiotics and multiple classes of agrochemicals. This locus features a naturally occurring allelic series—M (ancestral and non-resistant), A, AA, BA, and BP—characterised by increasing expression and insecticide resistance. Notably, the A, AA, and BA alleles likely predate the widespread use of agrochemicals following World War I, indicating that the locus has remained polymorphic prior to insecticide pressure. Our investigation of 18 wild populations along the eastern Australian coastline revealed that all allele forms, including the susceptible M, persist at varying frequencies. A leading explanation for this polymorphic maintenance is balancing selection, particularly due to intralocus sexual conflict (ILSC), where traits beneficial in one sex are detrimental in the other. Previous work showed that the BA allele increases female fertility but reduces male courtship success and aggression. While it is known that increased Cyp6g1 expression in the allelic series results from the presence of transposable elements and gene duplications, neither the native function of the Cyp6g1 gene nor the mechanisms behind ILSC and existing polymorphism are well understood in the fly model. Here, we examined the molecular mechanisms behind the effects of the M and BA alleles on fly fitness. We combined multi-omics analyses (transcriptional and metabolic profiles) with behavioural and fitness assays. To investigate this, we introgressed the M and BA alleles into a common genetic

background, isolating variation to the Cyp6g1 locus. At 25 °C, BA females produced twice as many offspring and developed significantly faster than M females. RNA-Seq analysis of BA versus M females revealed increased expression of Cyp6g2, a paralogue of Cyp6g1 recently identified as a key gene in Juvenile Hormone (JH) biosynthesis. As JH is known to promote female fertility but suppress courtship behaviour in young males, we hypothesised that Cyp6g1 exerts its pleiotropic effects via Cyp6g2 regulation. Using RT-qPCR, we confirmed that elevated Cyp6g1 expression correlates with increased Cyp6g2 expression in multiple introgressed lines and wild-type populations that also show changes in fitness. Using LC-MS to investigate metabolic profiles, we found that BA females had higher levels of JH (JH3) than M females, providing further support for our hypothesis. Furthermore, BA flies exhibited metabolic signatures consistent with enhanced energy and amino acid metabolism, while M flies showed signs of neural suppression. These findings suggest that the sexually antagonistic effects of Cyp6g1 are at least partly mediated by its influence on Cyp6g2 and downstream hormonal and metabolic pathways. While further investigation is needed to understand Cyp6g1–Cyp6g2 regulation of male fitness and behaviour, our study provides novel insights into the molecular mechanisms linking insecticide resistance with life-history traits and potentially the maintenance of Cyp6g1 polymorphisms in wild fly populations.



The genomic basis and evolution of immune trait variation in Soay sheep

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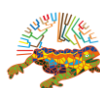
POSTER SESSION 3 - **P03.221**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Immune responses involve trade-offs between infection resistance and fitness traits such as reproduction and survival. Quantitative genetic theory predicts that strong selection will lead to a reduction in genetic variation for immune traits, yet often this variation persists. Investigating the direct relationship between genetic variation and fitness traits will help us to better understand this phenomenon of maintained variation and explore the potential role of balancing selection. Wild Soay sheep on St Kilda, Scotland, are naturally infected with the common gastrointestinal parasite *Teladorsagia circumcincta* (*T. circ*). Sheep with higher levels of the anti-*T. circ* antibody IgA have reduced parasite burden, yet there remains remarkably high phenotypic and genetic variation underlying the IgA levels within the population (heritability $h^2 = 0.57$, evolvability $I_a = 0.38$).

We determined the genetic architecture of IgA using 6,543 measurements from 3,190 sheep by integrating genomic data from 420K SNPs (all sheep) and whole genome

sequence data ($N = 134$ sheep). We used genome-wide association studies (GWAS) to confirm that IgA variation is largely underpinned by two major effect loci, the B-Cell Maturation Antigen (TNFRSF17) and the immunoglobulin heavy chain (IGH) complex. We examined associations between TNFRSF17 and IGH genotypes and four key fitness measures: juvenile survival, adult annual survival, adult annual reproductive success and adult annual fecundity. Interestingly, we found no association between these genotypes with fitness measures within this population.

Despite the persistence of genetic variation in anti-*T. circ* IgA, we find no evidence that this is maintained by a fitness advantage or balancing selection, suggesting other evolutionary forces such as drift may be at play. This study highlights the importance of using wild datasets for investigating immune variation's genetic basis, evolutionary role and the selective pressures shaping immune traits in natural populations.



Trans-species polymorphisms and long-term balancing selection across the primate radiation.

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POSTER SESSION 3 - P03.235, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Balancing selection is one of the rare evolutionary processes that can maintain, at intermediate frequencies, nucleotide polymorphisms that arose in the common ancestor of divergent lineages. Studying such trans-species polymorphisms could shed light into regions of the genome whose functional importance has been shared across multiple species. In primates, studies comparing hominid species have demonstrated that polymorphisms within immune-related loci are shared between human, chimpanzees and bonobos, and maintained at intermediate frequencies; and polymorphisms within the Major Histocompatibility Complex (MHC) have been reported in species having diverged more than 35 million years ago. Interestingly, a number of polymorphisms falling in non-coding regions have been described as shared between humans and chimpanzees, suggesting that perhaps balancing selection has targeted regulatory regions. Nevertheless, confounding factors such as recurrent mutations and duplications could still produce the observed shared polymorphisms, and studies accounting for these factors have only focused on specific regions of the genome and only in very few

species. Here, we propose to leverage the massive sequencing effort undertaken by the Marques-Bonet laboratory (consisting of more than 1800, 30x coverage, high quality genomes from species across the whole primate radiation) to evaluate long-term balancing selection in 24 non-human primate species from 6 different families. This work will rely on a strategy derived from previous work to analyze trans-species polymorphisms in human and chimpanzees, while also accounting for confounding factors through the use of stringent filtering criteria and per-species confirmation of local balancing selection genomic signatures. Preliminary results from an analysis that includes humans, chimpanzees and gorillas replicates some previously described polymorphisms, outside the MHC region, as well as previously unidentified polymorphisms in genes involved in functions ranging from neural development and cardiac to the immune system. Our study will thus extend our knowledge on both regions targeted by long-term balancing selection across the genome and the extent to which this evolutionary process has impacted the evolution of primates.



Using experimental evolution to explore patterns of dominance in *Drosophila melanogaster*

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POSTER SESSION 3 - **P03.241**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

We have previously carried out a female-limited X chromosome inheritance experiment in *Drosophila melanogaster*, with the aim of investigating standing sexually antagonistic variation on the X. Classical predictions from theory suggest that X-linked sexually antagonistic loci should exhibit specific patterns of dominance (e.g. female-benefit/male-detriment alleles should be preferentially dominant in females when located on the X). As part of our experimental design, we therefore always carried out assays on two types of female genotypes from the selection regime, either homozygous for the evolved X and autosomes, or heterozygous with a control X and autosomes making up half the genetic complement. This enabled us to investigate

signatures of additive or dominance variation for fitness between these genotypes, along with a number of other phenotypic traits. Using this unique dataset collected over 70 generations of experimental evolution, we found that some traits, such as body size, showed a consistent pattern of dominance, while others, such as fitness, showed evidence of overdominance (i.e. balancing selection). Interestingly, some traits seemed to initially show evidence of additivity, but when analysed per replicate line it became clear that the pattern of dominance/recessivity was variable among lines. Finally, we also have expression data for all female genotypes, which will enable us to detect more general patterns of non-additivity throughout the genome.



Antagonistic selection and genetic drift in eusocial ant colonies

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MEETING ROOM 122+123, August 20, 2025, 10:30 AM - 12:30 PM

Queens and workers in eusocial ant colonies exhibit very different phenotypes, ranging from large, winged, reproductive queens to much smaller, wingless, non-reproductive workers. Consequently, a mutation that improves queen performance may not improve worker performance, resulting in caste-antagonistic selection. Moreover, because the majority of colony members do not reproduce, the effective population size is significantly reduced, making genetic drift an equally important evolutionary force. The relative importance of selection and drift also depends on particular features of the eusocial species, such as whether the queen mates with multiple males (polyandry). While worker-biased selection is weaker under polyandry, the effective population size is larger, making genetic drift weaker as well.

In this talk, I will address how the population genetics of colony-structured eusocial populations is shaped by antagonistic selection and genetic drift, while accounting for various colony life-history traits, such as the number of queens per colony (monogyny

vs. polygyny), the number of mates per queen (monoandry vs. polyandry), queen survival probability, colony extinction probability, and migration probability. Specifically, I compute the fixation probability of mutant alleles in subdivided populations as a function of these colony life-history traits, with the goal of understanding the adaptive evolution of eusocial ant colonies.

Our results provide interesting insights. For instance, the effective population size decreases with increasing colony extinction probability, whereas polyandry has a relatively smaller impact on it. The probability of fixation of alleles that improve worker phenotypes is higher in polygynous ant colonies, and increasing the migration probability increases the probability of fixation for queen-beneficial alleles but decreases it for worker-beneficial alleles. Together, these results will help in the broader understanding of the role of genetic drift in the evolution of species with adaptive polymorphisms.



Are mitochondrial sisters their own worst enemies? Testing for negative frequency-dependent selection via resource competition in Trinidadian guppies.

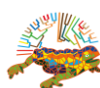
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MEETING ROOM 122+123, August 21, 2025, 10:30 AM - 12:30 PM

If an individual's niche is determined by its genotype, then competition for limiting resources should be most intense among individuals that share the same genotype. This is the crux of R. C. Lewontin's assertion that "a genotype is its own worst enemy". If true, Lewontin argued that this would generate negative frequency-dependent selection, contributing to the maintenance of genetic variation. Support for Lewontin's idea can be found in experimental studies of competition among clones of grasses, and in the maintenance of mitochondrial polymorphisms in laboratory populations of seed beetles and fruit flies. However, whether this mechanism operates under natural conditions is unclear. Using pedigree and individual demographic rates data from our long-term mark-recapture study of Trinidadian guppies, we asked whether females from the same matriline (i.e. mitochondrial sisters) competed more strongly with one another than with females from different mitochondrial backgrounds. Our dataset included ~20k observations of ~4k individuals over a period of 7 years and ~14 generations. We fit generalised linear mixed models for individual growth, reproduction, and survival, each as

functions of the number of mitochondrial sisters and the number of females from different mitochondrial backgrounds. We found that the negative effect of competition with mitochondrial sisters was twice as strong as that with females from different mitochondrial backgrounds. We then asked whether this heightened competition among sisters acted to maintain mitochondrial genetic variation within our study population. To do so we constructed a null simulation, where we simulated the pedigree while randomising births and deaths with respect to mitochondrial background. We then compared the rate of decline in mitochondrial diversity due to drift in this null model with the observed decline in mitochondrial diversity in our study population. Our results suggest that competition among mitochondrial sisters acted to decrease the rate of loss of mitochondrial genetic diversity in our study system. In conclusion, our results (i) imply that mitochondrial diversity can play a role in ecological competition, and (ii) demonstrate that competition among relatives can generate negative frequency-dependent selection, helping to maintain mitochondrial polymorphisms in a natural system.



Characterizing the detectable and invisible fractions of genomic loci under balancing selection

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MEETING ROOM 122+123, August 21, 2025, 10:30 AM - 12:30 PM

Balancing selection is natural selection that maintains genetic variation within populations. Thus, by definition, it maintains genetic variation that affects the fitness of individuals and can be responsible for the maintenance of diversity in interesting phenotypes. Researchers have argued that balancing selection may be rare and/or transient, and therefore would not be very important as one of the mechanisms of maintenance of genetic diversity, compared to neutral processes or other forms of natural selection. In contrast to that argument, genomic scans that looked for signatures of balancing selection among human populations have found that a sizeable fraction of protein-coding genes (1 to 8%) may be under balancing selection. Here, we address this debate about the prevalence of balancing selection by asking: 1) what fraction of loci under balancing selection remain polymorphic in face of genetic drift? and 2) what fraction of loci maintained polymorphic by balancing selection can be detected by genomic scans? We use a combination of theoretical analyses and computer simulations to address these questions. Theoretical results show the range of selection parameters that can lead to the maintenance of

polymorphism, and the expected width of the genomic signatures of balancing selection (local excess of heterozygosity). To address the second question, we simulate genomic data under various balancing selection models (overdominance, antagonistic pleiotropy and sexual antagonism, each with a range of selection coefficients), and several combinations of population parameters such as effective population size, mutation and recombination rates, to characterize the detectability of balancing selection in each scenario. We simulate under combinations of parameter values that include human-like and *Drosophila melanogaster*-like conditions. We find that balancing selection is generally hard to detect, with more than 50% of targets of balancing selection missed by existing methods. We further describe how population parameters influence detection power, showing that species with a high ratio of recombination to mutation rates (such as *D. melanogaster*) might have a particularly large fraction of undetectable balancing selection. Our results suggest that balancing selection may be more prevalent than previously appreciated, and more powerful methods to detect it might unveil interesting loci.



Ecotype evolution in the deep sea

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MEETING ROOM 122+123, August 20, 2025, 10:30 AM - 12:30 PM

Segregating alleles in natural populations can be driven to fixation or loss by genetic drift or directional selection, or may be maintained in a polymorphic state by balancing selection. In this study, we focus on the evolutionary processes affecting non-synonymous variants at functional loci, as discovered from whole genome scans in a deep-sea fish species (*Coryphaenoides rupestris*) that lives across habitat zones ranging from ~200m to ~2000m depth. At each of these functional loci, one allele is predominant in the deeper water (>1600m). The functions associated with depth at these loci are primarily involved with development and morphogenesis. There is no neutral genetic differentiation by depth. Across a shallower depth range (750-1500m), we find that minor allele frequencies show a highly significant increase or decline progressively

across five defined age categories, not seen for control loci (not associated with depth). Catch per unit effort data indicates that while juveniles tend to be found at ~1000m, as adults they either stay shallow or move deeper. Despite showing no neutral genetic differentiation, shallow vs deep ecotypes also show morphometric and dietary differences. Together these data indicate that ecotypes segregate to different depths, depending on their genotypes, and that balancing selection is retaining genotypes and phenotypes needed for each depth range. This is supported by signals for long-term balancing selection at these loci (but not at control loci). Balancing selection retaining polymorphism among individuals in a panmictic population is theoretically well established, but not widely understood at the molecular level.



Experimental support for balancing selection maintaining sexually antagonistic genetic variation in a polygenic trait

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Sexually antagonistic selection (SAS), where the same allele has opposing fitness effects in males and females, can maintain genetic polymorphism via balancing selection. While its theoretical basis is well established, the role of SAS in shaping the evolution of polygenic traits and constraining genome-wide responses to selection remains poorly understood. Here, we used the evolve and resequence approach in the seed beetle *Callosobruchus maculatus* to test the genomic signatures of divergent selection on body size in the presence and absence of sexual antagonism, by comparing allele frequency changes under SAS and sex-limited selection (SLS) over ten generations of phenotypic evolution.

In *C. maculatus*, body size is a sexually dimorphic trait closely linked to fitness: larger females are favoured for increased fecundity, while smaller males benefit from enhanced agility and mating success. Our experimental design included the following replicated treatments: selection under three modes of SLS (increasing male size, decreasing male size, and increasing female size), SAS (simultaneously favouring larger females and smaller males), and a relaxed selection control. All regimes produced clear evolutionary changes, with SLS resulting in the greatest overall phenotypic divergence in both sexes, and SAS producing the largest increase in sexual size dimorphism.

At the genomic level, SLS led to strong and consistent allele frequency shifts across replicates. Principal component analysis (PCA) of high-quality SNPs segregating in the ancestral population supported this pattern, revealing a clear and expected separation of SLS lines along the primary axis of genetic variation, consistent with their phenotypic divergence. In contrast, lines under SAS, despite showing directional phenotypic change, remained genetically close to the ancestral population along the same axis. These patterns suggest that SAS on body size

imposes a strong constraint on genomic divergence.

To explore the basis of this constraint, we focused on SNPs most strongly associated with body size evolution, identified through their high loadings on the primary PCA axis. These loci were enriched for intermediate-frequency alleles and showed elevated Tajima's D in the ancestral population, consistent with long-term balancing selection. This supports the hypothesis that variants currently constrained by SAS have historically been maintained by fitness trade-offs between the sexes.

Functional annotation revealed enrichment of developmental pathways, particularly BMP signalling. One gene, *Tolloid*, a conserved component of the BMP growth factor signalling pathway, carried both regulatory and coding variants that responded divergently to the SLS treatments. Under SAS, these variants exhibited limited allele frequency shifts and were located in regions of elevated ancestral polymorphism, further supporting a role for balancing selection. *Tolloid* plays a key role in dorsoventral patterning, scaling embryonic development to the size of the eggshell. Its polymorphism suggests that variation in adult body size may originate in variation in egg size, which has diverged among our selection lines in parallel with adult size.

Collectively, these findings provide direct experimental evidence that SAS maintains adaptive genetic variation in body size by preventing the fixation of alleles that have opposite fitness effects in males and females due to their influence on size. While SLS drove efficient and consistent genomic divergence, SAS preserved ancestral variation at key loci, despite ongoing phenotypic change. This highlights how evolutionary conflict between the sexes can limit short-term genomic responses while maintaining standing variation with long-term adaptive potential.



Genetic architecture and the maintenance of variation by sexual selection in the guppy

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MEETING ROOM 122+123, August 20, 2025, 10:30 AM - 12:30 PM

Guppies are a long-standing model for the maintenance of adaptive polymorphisms. In this system, males with rare color patterns are thought to be preferred by females, who benefit by having sons with similar patterns as their fathers, resulting in negative frequency dependent selection and spectacular color variation. This model of sexual selection requires that paternal phenotypes are good predictor of the patterns of their sons. Using a series of neural nets to identify color pattern from 14,100 high-resolution images, coupled with controlled pedigrees and GWAS, we determined the inheritance and architecture of 15 individual orange and black colour ornaments in a population of guppies. We

found several Y-linked ornaments that were faithfully inherited through patriline. These are largely encoded by regions that have recently duplicated from the autosomes to the Y rather than regions of the Y that are homologous to the X. However, most ornaments had X or more complex autosomal inheritance consistent with threshold traits, and for these the paternal phenotype was a poor predictor of the pattern of his sons. We explore how sexual selection dynamics influence the genetic architecture of adaptive variation using individual-based simulations, and re-evaluate our understanding of how genetic variation is maintained in this classic system.



Genetic interactions shape the evolution of adaptive polymorphism under resource competition

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MEETING ROOM 122+123, August 20, 2025, 10:30 AM - 12:30 PM

Background: An important question in evolutionary biology is what maintains genetic polymorphism in natural populations. Traditional theories of balancing selection provide important insights, but an aspect that is rarely addressed is the interplay of complex genotype-phenotype maps and frequency-dependent interactions between phenotypes. Fitness landscape theory has advanced our understanding of how genotypes map to phenotypes or fitness, yet it is rarely applied to systems with frequency-dependent selection. On the other hand, adaptive dynamics provides a powerful framework for studying frequency-dependent selection among phenotypes, but does not capture genetic details like epistasis, that is interactions between genetic loci. Bridging the gap between these approaches can help us develop a more complete understanding of adaptive polymorphism.

Results: We present a new theoretical framework that brings these two perspectives together. Our goal is to determine how communities of individuals with different genetic makeups evolve under complex interactions at the population scale. We introduce a tool called a set transition graph: Each node in the graph represents a set of genotypic subpopulations that can stably coexist under frequency-dependent selection. Edges in the graph show how one set can evolve into another through natural selection acting on a de novo mutation arising in the population. Nodes with no outgoing edges represent mutationally robust states that are local evolutionary endpoints in the regime of strong selection and weak mutation. The set transition graph gives us a bird's-eye view of the possible evolutionary routes that an evolving population might take.

We took this approach to study how competition for multiple resources can lead to variation

within a species. We used a model that combines two elements: A chemostat-like model describing how individuals compete for resources, and a map that links genotypes to traits quantifying the rate of uptake of those resources. Populations that are stable under resource competition form the nodes of the graph, which can be either monomorphic (single genotype) or polymorphic (multiple stably co-existing genotypes). An edge between two nodes – representing the effect of a mutation – may be associated with an increase or decrease in polymorphism, or simply the replacement of one genotypic subpopulation by a different one. We find that the model generates smoother transition graphs with longer adaptive paths to mutationally robust populations compared to analogous fitness landscape models without frequency-dependent interactions. We also show how epistasis increases the proportion of mutationally robust polymorphic states relative to monomorphic ones, thus promoting the emergence and persistence of adaptive polymorphism under resource competition.

Conclusions: Our work unifies genetic and ecological perspectives by embedding evolutionary dynamics into a set transition graph that captures the effects of both genetic and population-level interactions. We have used it specifically to understand how adaptive polymorphism emerges and persists under competition for multiple resources. Importantly, our work takes a step toward a broader synthesis between the graph-theoretical aspects of fitness landscapes and the ecological approach of adaptive dynamics, providing a more comprehensive picture of eco-evolutionary processes.



How do we resolve sexual antagonism? - An integrative approach to understand the genetic, developmental and regulatory basis of a sex-linked color polymorphism

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MEETING ROOM 122+123, August 21, 2025, 10:30 AM - 12:30 PM

Background: Color pattern polymorphisms have been a great model to understand the emergence and maintenance of genetic variation. A frequently observed polymorphism in African haplochromine cichlid fishes is the so-called blotched phenotype where females of certain populations in the radiations of Lake Malawi and Victoria develop irregular and unique patterns of black spots, compared to their regularly patterned male conspecifics. In cichlid species of Lake Malawi, the blotched phenotype has been associated with a cis-regulatory mutation around the *pax7a* locus, while in Lake Victoria it has been linked to a different chromosome. It remains unclear if the phenotype is shaped by similar, convergently evolved molecular mechanisms.

Materials & Methods: Our focal species is the Lake Victoria cichlid *Haplochromis chilotes*, where females of a population develop the blotched phenotype. We used a combination of high-fidelity long read sequencing (n=4) and short read whole genome sequencing (n=151) to investigate the genetic basis of the blotches. Additionally, we performed single-cell RNA sequencing (n=4) of adult fish and spatial RNA sequencing of embryos (n=2) to understand transcriptional differences between phenotypes. We used scanning electron microscopy, cell tracking during development and color pattern analysis to

better understand the tissue composition and cellular behavior across time and space. **Results:** We found a large heterozygous inversion on chromosome 16 in blotched fish associated with sex and blotch phenotype in *H. chilotes*. Our single-cell RNA sequencing moreover revealed that blotched fish have higher numbers of iridophores, accompanied by differential expression of various cell autonomous and non-autonomous factors linked to pigment cell differentiation and migration. The morphological analysis revealed that in embryos, melanophores that form blotches migrate faster and further, which in adults leads to large iridophore and melanophore aggregates in various layers of the skin.

Conclusion: The inversion harboring the W and the blotched loci in close linkage suggests the emergence of a new sex determining region on chromosome 16 in *H. chilotes*. Our results suggests that the genetic basis of blotches in Lake Victoria cichlids seems to have evolved via non-parallel genetic, molecular and mechanistic pathways. Nonetheless, the recurring patterns of the evolution of new sex determining regions in linkage with a pigmentation locus suggests that this mechanism could be a more widespread solution to resolve sexually antagonistic selection between nuptial coloration and camouflage patterns.



Larger environmental fluctuations at low frequencies can accelerate population recovery after abrupt environmental shifts by increasing genetic diversity, but this may occur along more vulnerable trajectories

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Background: Heritable variation for fitness plays a pivotal role in adaptive evolution. Among the mechanisms that may support its maintenance, temporally varying selection is especially important to study in the context of environmental change. Consider the effect of climate change on the Mediterranean Sea for instance. In addition to an increasing temperature trend, differential warming in summer vs. winter induces larger seasonal fluctuations, while marine heatwaves last longer and are more frequent. By modifying temperature-induced fluctuating selection regimes, climate change can alter the amount of heritable variation, and in turn, the ability of populations to cope with shifting means via adaptive evolution. Beyond climate change, this rationale conceptually applies to any change affecting both the mean and variance of the temporal distribution of environmental parameters.

Aim - Material & Methods: We undertake a theoretical approach based on the formulation of a simple minimal population genetics model that allows a rather general perspective. Within a finite diploid population, we consider a quantitative trait under non-optimal selection with a cycling optimum capturing environmental fluctuations. The trait is determined by a few dozen additive loci, and new alleles are continuously generated by mutations. We study the eco-evolutionary dynamics under contrasting regimes of environmental fluctuations (amplitude x frequency), and following an abrupt shift in the mean environment occurring in distinct background fluctuation regimes. We are interested in (1) the effect of environmental fluctuations on the dynamics of genetic diversity, (2) the implications in terms of resilience to abrupt environmental shifts, and (3) the genetic signature associated with fluctuating selection.

Results: We find temporally fluctuating selection to indeed be balancing with an increase in polymorphism and genetic variance. Additive

genetic variance oscillates around an average which can be several times that maintained in a constant environment. The higher averages correspond to large and slow environmental fluctuations, but the relationship shows an interactive effect of the amplitude and frequency as well as non-linear patterns. Mean trait and fitness also display cycling dynamics. When genetic variance is higher, the mean population trait tracks the environmental optimum more closely, mitigating the oscillations in fitness and population density.

Immediately after an environmental shift, the rate of adaptive trait change is much higher in the fluctuating regimes with higher genetic variances. In the long term, population fitness tends to recover faster in those regimes, but it goes through lower minimums associated with a higher extinction risk. At the genetic level, the increase in genetic variance is driven by a higher variance of allelic effects, while negative linkage disequilibrium also increases. Decomposing the genetic signal across loci reveals that most genetic variance is carried by only a few loci that are continuously renewed.

Conclusion: Because temporally varying selection (partly) maintains the genetic diversity required for evolutionary adaptation, we show that altering temporal variability patterns affects population recovery after a disturbance. Large environmental fluctuations might accelerate recovery, but at the cost of more vulnerable trajectories. Slow fluctuations are moreover required, in contrast with the faster rates that are overall projected.

The link between fluctuating selection and eco-evolutionary resilience further emphasizes the benefit of better detecting this type of selection in natural populations. Likely resulting from diminishing-return epistasis, we find an asymmetrical and dynamic distribution of the genetic signal across loci, that might prove relevant in that respect.



Protandry influences the maintenance of sex-limited polymorphism by antagonistic pleiotropy in the wood tiger moth

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MEETING ROOM 122+123, August 21, 2025, 10:30 AM - 12:30 PM

Balancing selection emerging from fitness trade-offs, generated by for example antagonistic pleiotropy, is one of the key mechanisms known to maintain genetic variation. Multiple theoretical models have shown that antagonistic pleiotropy can produce protected polymorphism, but only when selection is strong and with reversal of dominance, where heterozygotes express the allele with the highest fitness. The conditions for stable polymorphism become even stricter under sex-limited polymorphism, as only one sex experiences selection on the traits. We investigate how antagonistic pleiotropy between survival and reproductive success can maintain sex-limited polymorphism when there is a difference in time of appearance between sexes. Protandry, where males emerge earlier than females, is common among Lepidoptera, driven by competition among males for access to females. Here, we study protandry as a potential mechanism to increase selection on survival, thus

expanding the parameter space for polymorphism under antagonistic pleiotropy, in the model species wood tiger moth (*Arctia plantaginis*), with sex-limited color polymorphism. Male wood tiger moths are either white or yellow in hindwing coloration, and previous studies have demonstrated a trade-off between survival and mating success between the morphs, where yellow males are better protected against visual predators, but white males have a higher mating success. By applying an analytical and an individual-based model with realistic parameter estimates, we show that protandry can enable antagonistic pleiotropy to maintain polymorphism, as some degree of protandry is required for polymorphism to be stable in the wood tiger moth. Protandry also expands the dominance parameter space for polymorphism, where dominance reversal may not be required for protected polymorphism.



Sex-specific overdominance for reproductive fitness at a large-effect maturation locus in wild Atlantic salmon

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MEETING ROOM 122+123, August 20, 2025, 10:30 AM - 12:30 PM

The importance of balancing selection in maintaining variation in fitness-related traits, which are expected to be under strong selection, is a long-standing topic in evolutionary biology. Males and females share many traits that have a common genetic basis; however, selection on these traits often differs between the sexes, leading to sexual conflict. Under such sexual antagonism, theory predicts the evolution of genetic architectures that resolve this sexual conflict. Yet, despite intense theoretical and empirical interest, the specific loci underlying sexually antagonistic phenotypes have rarely been identified, limiting our understanding of how sexual conflict impacts genome evolution. Atlantic salmon is an interesting model system to address these challenges, as male and female Atlantic salmon have different optimal life history strategies, including differences in maturation timing and reproductive fitness and a large-effect locus linked with maturation age has earlier been identified.

Here, we construct a multigenerational pedigree to investigate sex-specific selection

on the large-effect maturation gene, *vgll3*, in a wild Atlantic salmon population. The *vgll3* locus is responsible for ~40% of the variation in maturation (sea age at first reproduction). Genetic parentage analysis was conducted on 18,265 juveniles (parr) and 685 adults collected at the same spawning ground over eight consecutive years. Sex-specific patterns of reproductive fitness were related to *vgll3* genotype. Females showed a pattern of overdominance where *vgll3**EL genotypes had three-fold more total offspring than homozygous females. In contrast, males demonstrated that late-maturing *vgll3**LL individuals had 2-fold more offspring than either *vgll3**EE or *vgll3**EL males. Taken together, these data suggest that balancing selection in females likely contributes to maintenance of variation at this locus via increased fitness of iteroparous *vgll3**EL females. This study demonstrates the utility of multigenerational pedigrees for uncovering complex patterns of reproduction, sex-specific selection, and the maintenance of genetic variation.



The micro- and macroevolutionary predictability of female-limited phenotypic polymorphisms

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¹Department Of Biology, Lund University

MEETING ROOM 122+123, August 20, 2025, 10:30 AM - 12:30 PM

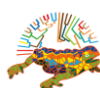
There is growing interest in the genomic and developmental basis of discrete and striking phenotypic polymorphisms in the animal kingdom. Recent research has uncovered the genomic basis of such polymorphisms and their maintenance over micro- and macroevolutionary time scales, particularly the role of balancing selection, such as negative frequency-dependent selection (NFDS). Such morphs often emerge through structural genomic variants, such as chromosomal inversions or through mutations in regulatory genes, sometimes with an additional role for introgressive hybridization.

Traditionally, researchers have focused on male-limited phenotypic polymorphisms and intrasexual competition among males for access to females as the ecological causes of NFDS. More recently, researchers have become aware of female-limited phenotypic polymorphisms that are also common, with sexual conflict, social selection or natural selection for mimicry maintaining such morphs. However, significant gaps in our understanding of female-limited phenotypic polymorphisms likely still linger as a historical legacy of the traditional focus on male conspicuous variation in colour and morphology.

Animal groups where such female-limited polymorphisms have been discovered and

described include insects like bedbugs, butterflies, damselflies and fruit flies but also vertebrates like birds and reptiles. In this talk, I will discuss the accumulating empirical evidence for female-limited phenotypic polymorphisms, their genomic basis, microevolutionary dynamics and macroevolutionary diversification across phylogenetic time scales.

I will illustrate my talk by also presenting some recent results from my lab on the genomic basis, micro- and macroevolutionary dynamics of female-limited colour polymorphisms in damselflies (Odonata: Coenagrionidae), with a special focus on forktail damselflies (genus *Ischnura*). These polymorphisms show surprisingly strong and predictable evolutionary dynamics, across both micro- and macroevolutionary time scales. Sexual conflict through pre-mating harassment by males on females play a key role in the maintenance of these polymorphisms, the conflict is also modulated by local ecology, including habitat openness, latitude, temperature, population density and local population genetic composition. Furthermore, plastic male mate preferences and male colour vision enabling female morph detection play a key role in maintaining these polymorphisms in natural populations.





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S49 - Time-dependency in micro- and macroevolutionary rates



Bioinformatic bias in pedigree-based mutation rate estimates: insights from sharks and whales

Dwivedi N¹, Scharf M^{3,4}, Naylor G⁵, Mona S^{1,2}

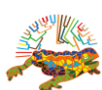
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POSTER SESSION 2 - **P02.144**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

All genetic variation ultimately stems from mutations introduced during DNA replication. The rate at which these mutations occur and get fixed is central to models of molecular evolution, enabling historical events to be dated and genetic diversity to be translated into population size estimates. Substitution rates can be estimated via phylogenetic methods, which compare sequence divergence from a known calibration point, while mutation rates can be estimated via direct approaches that involve whole-genome sequencing of pedigrees to identify denovo mutations. A longstanding paradox in the field is that mutation rate estimates are often lower than substitution rates, despite theoretical expectations that they should be higher. In theory, pedigrees should offer more reliable estimates, as they capture denovo mutations without the confounding effects of evolutionary forces that act over multiple generations and influence phylogenetic estimates. To explore the discrepancy between theoretical expectations and estimated mutation rates, we examine how bioinformatic pipelines influence denovo mutation detection in pedigrees. We focus on bioinformatic pipelines, as a prior study by Bergeron et al. shows that different research groups analyzing the same pedigree data can produce a two-fold difference in the mutation rate estimate [eLife, 11, 73577 (2022)]. We use two case studies: the epaulette shark, recently reported to have the lowest vertebrate mutation rate (7×10^{-10} per site per generation), and the humpback whale. Applying each species' pipeline to the other results in significant shifts in mutation rate estimates for both species, with a two-order magnitude increase in the epaulette shark's mutation rate and a one-order decrease in the humpback whale's. This discrepancy arises from specific filtering steps, such as quality-by-depth thresholds, which influence the pipeline's ability to distinguish denovo mutations from sequencing errors, especially when pedigree samples are sequenced at heterogeneous depths.

To assess whether the bioinformatic pipelines provide an unbiased view of genome evolution, we sequenced eight individuals from a natural epaulette shark population at 27X coverage. Three lines of evidence suggest that the bioinformatic pipeline used to estimate the mutation rate targets non-random genomic regions enriched for conserved elements. First, assuming the Wright-Fisher reproduction model, the estimated mutation rate and genome-wide nucleotide diversity of 1.3×10^{-3} per site observed in the natural population imply an effective population size of ~ 1 million. Although the relationship between census and effective population size is complex, such high values are unrealistic for a geographically restricted species. Second, the callable genome (i.e., regions passing filters in the pedigree analysis) accounts for 90% of annotated genes despite representing only 27% of the total genome size. Third, nucleotide diversity within the callable genome is 1.6 times lower than the genome-wide average with an excess of low-frequency variants, as indicated by a negative Tajima's D of -0.17 (contrasting with the genome-wide Tajima's D of 0.32).

Our findings underscore how methodological choices, particularly variant filtering, can bias pedigree-based mutation rate estimates. We caution against universal mutation rate assumptions and advocate for generating species-specific estimates tailored to genomic regions that experience distinct selective pressures. More broadly, we highlight how combining sequencing data of differing qualities can introduce bias during genotype filtering, an important consideration in any study involving pooled libraries. By comparing pipelines and evaluating their impacts on the population genetic statistics of natural populations, we aim to improve the consistency of pedigree-based mutation rate estimates and reconcile them with evolutionary theory.



Conflicting Timelines: Exploring patterns of divergence discordance across data types among tetrapod groups

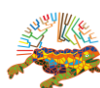
Ranganathan E¹, **Karanth P**¹

¹Indian Institute Of Science

POSTER SESSION 2 - **P02.152**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Divergence estimates across tetrapod groups vary substantially depending on whether mitochondrial, nuclear, or a mix of concatenated mito-nuclear markers are employed, yet the extent and causes underlying this discordance remain under-explored. While previous studies have examined divergence disparities in smaller taxonomic groups, a broad comparative analysis across tetrapods is still lacking. Here, we quantify divergence disparities across primates (Order Primates), birds (Class Aves), squamates (Order Squamata), and anurans (Order Anura) while controlling for calibration strategies, taxon sampling, and other a priori distributions. We also assess substitution saturation across data types to elucidate its role in generating unreliable divergence estimates. Our results show that mitochondrial markers

consistently underestimate basal divergence times and overestimate recent divergences relative to nuclear datasets, except in anurans. Furthermore, estimates from concatenated mito-nuclear alignments tend to skew toward nuclear estimates across groups. While mitochondrial datasets exhibit strong substitution saturation, we also detect saturation in the extensive anuran nuclear dataset, reducing mito-nuclear divergence disparities in their crown ages. These findings highlight regions of pronounced divergence discordance across tetrapod phylogenies, and emphasize the need to reassess divergence estimates based on saturated data. Additionally, our results reveal a nuclear bias in concatenated mito-nuclear estimates, reinforcing the importance of data choice for molecular dating.



Idiosyncrasies unveiled: examining the pace, patterns and predictors of biotic diversification in peninsular India

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POSTER SESSION 2 - **P02.182**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Diversification, a balance between speciation and extinction, governs biodiversity patterns and the hyperdiverse tropical biome has been central to diversification studies. Recent studies across tropical regions suggest that patterns of diversification depend on regional biogeographic and geoclimatic processes, geographic settings such as being an island or a continent, and vary across the Tree of Life. Therefore, identifying and characterising the processes responsible for uneven species diversity among distinct geographical regions and clades is essential for a nuanced understanding of the factors contributing to tropical diversity.

In this regard, the Peninsular India Plate (PIP) remains relatively unexplored despite being one of the oldest regions for diversification in tropical Asia. The PIP was a part of the Gondwanaland supercontinent and drifted in isolation for nearly 50 million years before finally colliding with Asia, leading to the formation of the Himalayas. Owing to this, the landscape experienced a drastic latitudinal shift leading to geo-climatic and habitat changes that influenced the biotic diversification in the landscape. Today, the PIP harbours a highly diverse biota with varying biogeographic affinities, including Gondwanan and Asian. Many of them have been reported to have formed endemic radiations through in situ diversification. However, our understanding of the diversification dynamics of its biota within a quantitative framework remains limited. In this study we build on the detailed phylogenetic and biogeographic studies of the endemic PIP biota to infer diversification rates, patterns, and potential drivers of diversification within the landscape.

Materials and Methods: We used time-calibrated molecular phylogenies and birth-death models to examine the tempo, mode, and drivers of diversification across 33 well-studied lineages (~770 species) which are highly endemic (> 67 %) to the PIP. We inferred the mean rates (across

time) and temporal trends in diversification using the time-varying birth-death models from the R package RPANDA, the ClaDS model from the Julia package PANDA and the CoMET (CPP on Mass Extinction Times) analysis from the R package TESS. Furthermore, we assessed the relative influence of the standing species diversity through time, paleotemperature, himalayan orogeny and expansion of C4 plants in the PIP on the diversification rates using the diversity-dependent and environmental-variable-dependent birth-death models, respectively.

Results: Among the PIP lineages, angiosperms diversified the fastest, invertebrates the slowest and younger lineages of Asian origins diversified more rapidly than the older relictual Gondwanan lineages. Evolutionary relatedness explained the disparities in diversification rates across taxonomic groups and biogeographic origins. A gradual accumulation of diversity was supported in 17 lineages and the remaining 16 lineages favoured diversification slowdowns and diversity declines. A high prevalence of gradual accumulation suggests that the historical stability of their habitat was an important driver. Furthermore, for the remaining lineages, the temporal changes in the diversification rates were majorly explained by the Miocene intensification of monsoons and aridification and fluctuations in paleotemperature.

Conclusion: We show that diversification in the PIP has signatures of regional drivers like intensified mid-Miocene aridification and many known global drivers like tropical ecosystem stability and paleotemperature. The complex tempo and mode of diversification shown in our results suggest that regional biogeographic, geoclimatic and phylogenetic history are critical to understanding the origins of tropical diversity and need to be examined across tropical regions.



Introducing the early high disparity phylogenetic comparative model, with applications to ichthyosaur macroevolution and implications of sedimentary sequence biases

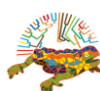
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¹University Of Copenhagen

POSTER SESSION 2 - **P02.040**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Early high disparity (EHD) is an evolutionary mode frequently encountered in the fossil record, describing a pattern of morphological disparity reaching a maximum in the early portions of a clade. Methods for modelling processes resulting in EHD patterns are currently lacking, leading to the development of a novel phylogenetic comparative method (PCM) presented here. This PCM combines two modes of evolution: the Ornstein-Uhlenbeck (OU) and Early Burst (EB). Individually, these two component modes display time-dependency where the EB mode represents decelerating rate in σ^2 , and the OU mode displays deceleration in the mean trait through time via the magnitude of α . Combined into a single mode, the EHD mode represented by EB and OU results in an explosive increase in disparity, reaching an early maximum disparity as a result of the EB parameters, thereafter decreasing in rates and disparity due to the constraining influence of the OU parameters. In macroevolutionary terms, this 'boom-and-bust' pattern of disparity may result from a clade initially undergoing an adaptive radiation, followed decrease in disparity due to stronger selective pressures towards a single adaptive optimum. Presented here are the theoretical statistics and model adequacy tests using Monte Carlo simulations to interrogate the performance of this method when the generating (simulating) modes are EHD and alternative modes (including OU and EB alone). To test the method on an empirical case, EHD model fitting is performed on ichthyosaur size datasets, a group of Mesozoic marine reptiles which seemingly display an EHD pattern. Compared to alternative evolutionary modes, the EHD mode is recovered as best fitting in nearly all ichthyosaur datasets tested ($\Delta AICc < 2$).

Across all model fits, the median expected time of peak disparity equals 231.1 Ma, occurring within the Triassic Period and approximately in the first 12.4% of ichthyosaur phylogenetic age. These results are robust especially when performing model adequacy tests based on the mean best fitting parameter estimates across all ichthyosaur trees, where EHD is often recovered as the best fitting mode when the simulating mode is also EHD (99% acceptance rate). Possible environmental factors driving an EHD mode in ichthyosaurs are investigated, along with the potential role for geological preservation biases in producing apparent EHD patterns. One such potential geological bias driving increased model performance of the EHD mode is sedimentary sequence duration. Using changepoint analyses for time series of Triassic Period sequence boundary ages, a significant changepoint is recovered occurring at 237 Ma, approximately six million years before the time of peak disparity for ichthyosaurs. Boundary ages of sedimentary sequences are sources for time-calibration of phylogenetic branches, therefore biases impacting sedimentary sequences translate to errors in phylogenetic branch length estimation. Rates tend to be elevated along shorter branches, likewise shorter sedimentary sequences may result in apparently elevated evolutionary rates if duration is not properly estimated after accounting for this effect. Therefore, elevated model support for the EHD mode in ichthyosaurs may ultimately result from geological biases such as sedimentary sequence duration, an example of an important bias in apparent time-dependency of macroevolutionary phenomena in paleobiological datasets.



Multiple processes contribute to time-dependency in contemporary rates of genome-wide evolutionary change

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POSTER SESSION 2 - **P02.064**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Lab, field, and fossil studies have shown that phenotypic evolution often occurs rapidly over short time scales but more slowly over longer time scales. These patterns may be explained by various forms of stabilizing or fluctuating selection. However, much less is known about the nature and causes of the time-dependency in genome-wide molecular evolution.

Materials and Methods: We estimate the rates and time-dependency of genome-wide evolution in natural populations of *Lycaeides* butterflies using a spatio-temporal genomic dataset spanning approximately 30 populations, over 10,000 butterflies, and more than 20 years (with >200 population × year combinations). Using pooled whole-genome sequencing data, we measure allele frequency changes across different temporal scales and spatial locations. We then quantify the time-dependency of allele frequency changes and test alternative models to explain these patterns.

Results: Analyses of our pooled whole-genome sequence data revealed that *Lycaeides* butterfly populations exhibit detectable genome-wide evolutionary change from generation to generation, with an average per-generation allele frequency change of approximately 0.01 (averaged across populations and SNPs). This level of change exceeds that observed in replicate samples from the same population and year, suggesting that we are detecting actual evolution (even if driven by drift) rather than sampling error alone. Importantly, we found a pronounced negative correlation between inferred rates of evolution (estimated allele frequency change) and the number of generations considered.

While much of the documented evolutionary change likely reflects genetic drift, we also detected additional patterns suggesting the involvement of selection and gene flow in explaining both the change and its time-dependency. For example, when focusing on 10-year intervals, we observed a preponderance of positive correlations in genome-wide allele frequency changes between population pairs. These correlations were stronger (larger and more positive) among nearby populations, consistent with the hypothesis that shared selection pressures and gene flow among geographically proximate populations contribute substantially to short-term genome-wide evolutionary change. Interestingly, such patterns were not observed when examining shorter time intervals of one or a few years, suggesting that very short-term changes are dominated by genetic drift, while the cumulative effects of selection and gene flow become detectable over longer timescales, such as decades.

Conclusions: In conclusion, we documented the time-dependency of genome-wide allele frequency change, with rapid evolution occurring over very short time scales and slower rates of change over longer, decadal time scales. We believe this pattern can be explained by a combination of genetic drift, gene flow, and fluctuating selection. These patterns differ from the more gradual, clock-like patterns of molecular evolution observed over much longer time scales, a discrepancy that we believe reflects the more reversible nature of allele frequency change (and quantitative trait evolution) relative to allelic substitution.



Numerous karyotype rearrangements of diptera evolution on various timescales.

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¹Wellcome Sanger Institute

POSTER SESSION 2 - **P02.049**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

The genomes of Diptera are considered to be evolutionarily stable, as demonstrated by the conservation of chromosomal arms between *Drosophila* and tephritid fruit flies. Nevertheless, *Drosophila* genomes also show high rates of chromosomal arm exchanges and frequent intrachromosomal inversions, showing the genomic stability operates only on specific aspects of genome organisation. To assess whether these evolutionary patterns hold across the whole dipteran order, we reconstructed the karyotype evolution using 288 chromosomal-level assemblies of diptera from 52 families. Contrary to the common expectation, we found that the Muller elements have evolved only in the common ancestor of Schizophora, dagger flies and

hoverflies and are stable since. However, the evolution of Muller elements are the only ancient fixed chromosomal rearrangements; all the sequenced nematoceran superfamilies retained the ancestral karyotype, suggesting that indeed, dipteran karyotypes are largely stable. However, there are some exceptions on the family level, with Chironomidae being the family with the most rearranged genomes, despite relative conservation of the other culicomorphan genomes. The inferred ancestral karyotype offers a novel perspective on genome evolution in Diptera, laying the groundwork for further investigations into evolution of chromosome rearrangements and sex chromosomes.



Speciation completion rates have limited impact on macroevolutionary diversification

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POSTER SESSION 2 - P02.163, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Standard birth-death (BD) processes used in macroevolutionary studies assume instantaneous speciation, an unrealistic premise that limits the interpretation of speciation and extinction rates. The protracted birth-death (PBD) model instead assumes that speciation involves two steps: initiation and completion. In order to understand their respective influence on macroevolutionary speciation rates, we compute a standard time-varying BD scenario that is 'equivalent' to the PBD model in terms of speciation and extinction probabilities. First, we find a sharp decline in the equivalent birth rate near the present, indicating that rates estimated at the tips of phylogenies may not accurately reflect the

underlying speciation process. Second, the completion rate controls the timing of the decay rather than the asymptotic equivalent rates. The equivalent birth rate in the past scales with the speciation initiation rate, with a scaling factor depending mostly on the population extinction rate. Our results suggest that the rates of population formation and extinction could often play a larger role than the speed of accumulation of reproductive isolation in modulating speciation rates. Our study establishes a theoretical framework for understanding how microevolutionary processes combine to explain the diversification of species on macroevolutionary time scales.



Temporal and non-temporal patterns in rates of diversification and phenotypic evolution in a hyperdiverse fish clade

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POSTER SESSION 2 - **P02.174**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Uncovering patterns of variation in evolutionary rates over time, how they relate to other factors – such as the presence of evolutionary innovations – and whether there is an association between rates of speciation and rates of phenotypic evolution is crucial. Indeed, this allows testing conflicting predictions from different models and, more generally, understanding how and why biological diversity varies greatly through time and space. Here, we investigate patterns of diversification and phenotypic evolution in wrasses, a hyperdiverse fish clade.

Materials and methods: We approached the problem of variation in evolutionary rates through an integrative approach combining multiple comparative methods. We first obtained a novel time-calibrated phylogeny of 380 wrasse species. Using this phylogeny, we fitted various models of diversification, including tree-wide models (both time-constant and time-dependent) and models allowing for lineage-specific or clade-specific rate shifts. We applied a similar modelling framework to phenotypic evolution in body size, shape, and elongation, by fitting both tree-wide models and models allowing for shifts. Finally, we tested for the association between rates of diversification and phenotypic evolution.

Results: Our diversification analyses supported overall increasing rates towards

the present (i.e., time-dependent diversification). Conversely, rates of phenotypic evolution showed no evidence of time-dependency. At the same time, we identified substantial variation in evolutionary rates across lineages and clades both for speciation and phenotypic evolution. Elongation was the only trait for which we found a clear association between speciation and phenotypic evolution, and even this pattern was not consistent across all lineages.

Conclusions: Our findings reveal complex temporal patterns in evolutionary rates within wrasses. Diversification analyses support a broadly time-dependent pattern with multiple superimposed lineage-specific shifts. In contrast, phenotypic evolution appeared to lack an overall time-dependency in rates but still showed clear shifts in rates superimposed on the general pattern. Notably, the only trait with a clear association between rates of speciation and phenotypic evolution was elongation – a specific aspect of phenotypic variation. Elongation has been repeatedly shown to be associated with microevolutionary ecological divergence (between benthic and limnetic forms) in several teleostean clades. Our results contribute to the ongoing debate on evolutionary rates by demonstrating complex, lineage-specific patterns superimposed on simpler global trends.



The origin and diversification of turmeric (*Curcuma*: *Zingiberaceae*) in paleotropical biodiversity hotspots: the role of ancient hybridization and historical climate change

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¹Institute of Botany of CAS, ²Charles University, ³National Museum, ⁴Singapore Botanic Gardens, ⁵National University of Singapore, ⁶Queen Sirikit Botanic Garden, ⁷Becamex Institute of Research and Development, ⁸Southern Institute of Ecology, ⁹Malabar Botanical Garden and Institute for Plant Sciences, ¹⁰Central European Institute of Technology, ¹¹Royal Botanic Garden Edinburgh

POSTER SESSION 2 - **P02.135**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Aim: To investigate the ecological and evolutionary mechanisms driving biodiversity in the Indo-Malayan hotspots, with a particular focus on the roles of ancient hybridization and historical climate in shaping species diversity.

Location: The Indo-Malayan region, particularly the Western Ghats and Indo-Burma biodiversity hotspots.

Time Period: The Miocene Climatic Optimum (~14 million years ago) to these days.

Major Taxa Studied: *Curcuma* (*Zingiberaceae*).

Methods: The evolutionary history of *Curcuma* was reconstructed using targeted nuclear loci.

This genomic data was integrated with ecological niche modeling, biogeographic reconstruction, and diversification dynamic analyses to examine the interplay between

climate stability, niche evolution, and hybridization.

Results: *Curcuma* originated in the Indo-Burma region ~14 Mya. Its diversification was primarily driven by long-term climatic stability and niche expansion, with dispersal to the Western Ghats occurring in the mid-Pliocene. While ancient hybridization likely facilitated ecological divergence and adaptation, it had no statistically significant impact on diversification rates.

Main Conclusions: Our work provides support that the Indo-Malayan biodiversity is a result of species accumulation enabled by climatic stability over evolutionary timescales, rather than increased speciation rates. These findings enhance our understanding of the processes shaping paleotropical biodiversity and offer insights relevant to the conservation and improvement of *Curcuma*, a crop of economic and medicinal value.



Uncovering dynamic adaptive landscapes on a phylogeny using the Ornstein-Uhlenbeck model

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POSTER SESSION 2 - **P02.138**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: The adaptive landscape of morphological traits changes across time and space. Under similar environmental and species-specific drivers, traits of distantly related species can share the same adaptive landscape. Conversely, changes in evolutionary drivers can lead to adaptive shifts, that is, events that modify the adaptive landscape of a species. Adaptive shifts can be categorized into two types: shifts in the adaptive peak (optimum shifts) and shifts in the rate of adaptation (rate shifts). Using the Ornstein-Uhlenbeck (OU) model, we can infer adaptive shifts on a phylogeny from observations of the continuous trait data alone. This is known as a hypothesis-free approach, where the occurrence of adaptive shifts signifies some underlying, unobserved drivers of evolution. Most commonly, the multi-optima OU model is employed to model optimum shifts. However, this model assumes a global rate of adaptation that is constant over time and across lineages. In other words, we cannot test whether or not adaptation is faster or slower for specific lineages or at specific times in the geological history under the current model.

Methods: We propose a novel Bayesian approach to infer adaptive shifts on a phylogeny using the OU model implemented in RevBayes. Our method has three main novelties. First, we derived a novel and efficient likelihood function and implemented it in RevBayes. Second, the model allows shifts in any combination of the OU parameters such that both optimum shifts and rate shifts can be inferred. Lastly, the number of adaptive peaks can be estimated in a Markov chain Monte Carlo

efficiently using a Dirichlet process prior (DPP). Using simulation studies, we assess our model in terms of its accuracy to infer (1) the locations of adaptive shifts and (2) the OU parameters for each adaptive peak. Specifically, we focus on the ability to distinguish different rates of adaptation when there exists one or more adaptive peaks. Lastly, we demonstrated our model empirically through exploring how the adaptive landscape of the visual system in birds changes over time.

Results: Simulation results show that our OU model can map adaptive shifts on a phylogeny accurately (if there is sufficient signal in the data), and different rates of adaptation can be robustly inferred. The model has larger power when the rates of adaptation are higher, possibly because the adaptive peaks are more distinct. The use of a DPP also allows us to infer the number of adaptive peaks without using a computationally intensive reversible-jump algorithm, or the need to go through model selection.

Conclusion: Overall, our novel method provides a biologically more realistic way to reconstruct the adaptive landscape across the phylogeny. Importantly, it is computationally efficient and can be applied to large phylogenies with thousands of species. We believe our approach can be widely used to study patterns of adaptation, especially dynamic rates of adaptation across time and species.

Keywords: adaptive landscape, Ornstein-Uhlenbeck model, phylogenetic comparative methods, RevBayes



Estimates of the Mutation Rate per Year Can Explain Why the Molecular Clock Depends on Generation Time

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MEETING ROOM 122+123, August 19, 2025, 11:00 AM - 1:00 PM

Rates of molecular evolution are known to vary across species, often deviating from the classical expectation of a strict molecular clock. In many cases, the rate of molecular evolution has been found to correlate to generation time, an effect that could be explained if species with shorter generation times have higher mutation rates per year. We investigate this hypothesis using direct estimates of the mutation rate for 133

eukaryotic species from diverse taxonomic groups. Using a phylogenetic comparative approach, we find a strong negative correlation between mutation rate per year and generation time, consistent across all phylogenetic groups. Our results provide a simple explanation for why generation time plays a pivotal role in driving rates of molecular evolution across eukaryotes.



Measuring morphological changes in unit of nucleotide changes to disentangle neutral and adaptive processes

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MEETING ROOM 122+123, August 19, 2025, 11:00 AM - 1:00 PM

Morphological and molecular rates of evolution are measured as the rate of change of a trait per unit of time, and can be measured over different timescales. However, there is a discrepancy between the rates of change measured over macro-evolutionary timescales and those measured over micro-evolutionary timescales. In fact, observed rates of changes are smaller than expected on longer time-scales, and whether this shift in rates reflect genuine biological processes or methodological artifacts is currently debated. Given this uncertainty, distinguishing different selection scenarios acting on morphological evolution is challenging since it therefore depends on the timescale of the analysis. Instead, to alleviate such ambiguities, we propose to investigate morphological rate of evolution as a function of nucleotide divergence rather than time. To showcase the potential of this approach, we present two applications. First, in a phylogenetic context, we can measure morphological changes on a tree

with branch lengths expressed in nucleotide divergence compared to a tree measured in time unit. By doing so, we can test for deviations from a neutral expectation and account for variation in generation time and molecular rates along the branches of the tree, absorbing the uncertainty in the molecular clock. Second, studying morphological changes as a function of nucleotide changes allows us to integrate together micro-evolutionary and macro-evolutionary processes in the same framework. More precisely, we can normalize between-species variation by nucleotide divergence, and within-species variation by nucleotide polymorphism. And together, we can derive a neutrality index as the ratio of between- to within-species trait variation. Such a ratio allows us to test for the deviation from the neutral expectations and detect adaptive processes. Altogether, by shifting the focus from time to nucleotide changes, we argue that we can disentangle neutral and adaptive processes acting on morphological evolution.



Rate-time scaling in phenotypic evolution: Limitations of current models in capturing temporal dynamics

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MEETING ROOM 122+123, August 19, 2025, 11:00 AM - 1:00 PM

Evolutionary rates correlate negatively with time, which makes it complicated to compare rates across lineages that diversified on different time intervals. The causes of this correlation are debated. In this study, we use evolutionary time series to investigate the effects of model misspecification, sampling error, and model identifiability on the relationship between rates of phenotypic change and time. Using simulations, we first show that rates of evolution estimated as a parameter in the unbiased random walk (Brownian motion) model lack a rate-time scaling when data has been generated using this model, even when time series are made incomplete and biased. This indicates that it is theoretically possible

to estimate rates that are not time correlated. We then analyze 643 empirical time series to assess whether accounting for model misspecification, sampling error, and model identifiability reduce the negative scaling, but none appear to have a significant impact. This suggests that the rate-time correlation requires an explanation grounded in evolutionary biology, and that common models used in phylogenetic comparative studies and phenotypic time series analyses often fail to accurately describe trait evolution in empirical data. Making meaningful comparisons of estimated rates between clades and lineages covering different time intervals remains a challenge.



The rise, decline and fall of clades

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MEETING ROOM 122+123, August 19, 2025, 11:00 AM - 1:00 PM

How and why species diversity varies over geological time scales remains disputed. We analyzed the evolutionary history of 27 radiations with phylogenetic information incorporating extinct and extant species under a new 'fossilized birth-death diffusion' model that provides a detailed characterization of past diversification and resulting diversity dynamics. Clade diversity trajectories follow rise and decline dynamics, with fast accumulation following recurrent speciation while slowdowns and

losses are modulated primarily by changes in extinction. Diversity dynamics do not appear to be governed by clade-level processes expected from adaptive radiations or diversity equilibria. Rather, these patterns emerge from dynamics at the species-level, where lineages tend to become increasingly vulnerable to extinction and less likely to speciate with time. Those species that counteract this trend create and maintain biodiversity through deep time.



The role of mutational neighborhoods and evolvability in shaping evolutionary tempo

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MEETING ROOM 122+123, August 19, 2025, 11:00 AM - 1:00 PM

Evolutionary rate estimates assume that mutations happen randomly and independently over time. This works for mutation appearance rates, but not for fixation rates. Some mutations can change traits like robustness or evolvability, which then affect future mutation fixation rates, violating the initial independence assumption. To test whether this mechanism is plausible, we used the Aevol artificial life simulation platform. Aevol is an individual-based simulator of molecular evolution in which populations of virtual organisms evolve through mutation and selection. Aevol can simulate complex mutational processes, including substitutions, duplications, indels, inversions, and translocations. It (1) guarantees that mutational events occur completely independently, and (2) enables the rate of mutation fixation to be measured over long periods of time, thus making it possible to verify whether or not the independence hypothesis holds true.

Using this platform, we first evolved 30 “wild-type strains” long enough to reach a quasi-stable state. We then cloned these strains 30 times each, resulting in 900 populations which were evolved further in the same constant environment. Since they were well adapted to this environment, most of these populations remained in evolutionary stasis all along the experiment, showing only rare fixation of neutral or quasi-neutral mutations. But in around 20% of populations, evolutionary dynamics shifted to a transitional state of accelerated evolution, during which the rate of mutation fixation increased substantially. These populations then returned to stasis, although a few showed secondary bursts. To understand what might trigger these accelerations in evolutionary rates, we

analyzed all events fixed in the lineages of these populations, quantifying, for each type of mutational event, the average contribution to fitness, robustness, and evolvability as well as the average waiting time until the next event is fixed. Results show that sequence duplications greatly reduce the average waiting time until the next event is fixed and greatly increase lineage evolvability. Further analyses highlight an important property of different types of mutational events that is generally overlooked by evolutionary theory: their combinatorics.

Combinatorics refers to the number of different genomes accessible through a specific mutation type. In other words, combinatoriality quantifies the size of a genome's mutational neighborhood for a given type of mutation. Strikingly, the combinatorics of rearrangements is far greater than that of simpler mutations (e.g., substitutions and indels). It immediately follows that the time needed to explore a substantial fraction of the mutational neighborhood varies by several orders of magnitude depending on the type of mutation. However, the combinatorics alone cannot explain the punctuated dynamics we observed in our experiments. Our results also show that some types of mutational events (typically duplications) increase the probability of fixation of others through an increase in evolvability. Hence, the punctuated evolutionary dynamics we observe in our simulations results from the interaction of different mutational processes, each with its own tempo.

Reference: Banse, P., Elena, S. F., and Beslon, G. (2024). Innovation in viruses: fitness valley crossing, neutral landscapes, or just duplications? *Virus Evolution*, 10(1), veae078.





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S50 - Unraveling the origin of eukaryotes: integrating prokaryotic and eukaryotic perspectives



Cyanobacterial Contributions to the Evolutionary Origins of Animals

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POSTER SESSION 3 - P03.244, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: The evolutionary emergence of animals remains a mystery [1]. Alongside all kingdom evolutionary studies aimed at a broad understanding of eukaryogenesis [2], researching the phylogeny of characteristic animal traits may give insights into our evolutionary origins. We build on observations from our labs [3] and others [4,5] that cyanobacteria have proteins surprisingly similar to chemical signalling-related proteins in animals.

Neurotransmission is a defining characteristic of animals. Most animals have some form of nervous system [6]. Elements of the nervous system pre-date the emergence of animals [7]. Nitric oxide [8] and retinoic acid (Vitamin A) [9] play roles in the nervous system [10] and have wider signalling functions that again are seen as distinctive animal features. Herein we present phylogenetic analyses of proteins involved in these pathways and in neurotransmission. We also present experimental data from our labs and others', that suggests that cyanobacterial homologs of key animal proteins have related biochemical functions.

Materials & Methods: Pilot trees were generated by web-based methods, to inform a HPC protocol interrogating the GTDB (Genome Taxonomy Database) using HMMER and aligning sequences with MAFFT. Phylogenetic trees were built using IQ-TREE and visualised using Interactive Tree Of Life (iTOL). Selected homologs were expressed in *E. coli* [11]. A putative retinal dehydrogenase from *Chlorogloeopsis fritschii* was assayed by mass spectrometry [11]. A putative *Okeania* sp. calmodulin-like domain was assayed using 1D 1H and TROSY NMR spectroscopy. Kinetics experiments for GCH1 and histidine decarboxylase homologs from *Thermosynechococcus elongatus* and *Nostoc linckia* are underway.

Results: Our published studies on homologs of retinal dehydrogenase, which biosynthesises retinoic acid, revealed a surprisingly recent common ancestor of animal and cyanobacterial enzymes [9]. With respect to nitric oxide and peroxynitrite signalling, our multiple sequence alignments of nitric oxide synthases and NOX5 homologs, plus a phylogenetic analysis of NOX5, also suggests eNOS and NOX5 in animals originated through horizontal gene transfer, as with retinal dehydrogenase, from ancestral

cyanobacteria [8]. Our experimental studies on a cyanobacterial retinal dehydrogenase homolog [11] and results from other laboratories on cyanobacterial NOX5 [12] and eNOS homologs [13] suggest common biochemical functions across kingdoms.

Our unpublished phylogenetic trees on the core machinery of calcium-mediated neurotransmission, ionotropic glutamate receptors (iGluR) and calmodulin, revealed similar close relationships between animal and cyanobacterial homologs. Our computational research based on experimental structures for cyanobacterial GluR0 [14], and NMR experiments confirming calcium binding in a cyanobacterial calmodulin homolog, again support equivalent biochemical functions.

Phylogenetic analyses of enzymes that underpin biosynthesis of neurotransmitters like serotonin and dopamine additionally suggest animals acquired these pathways from ancestral cyanobacteria. We have expressed selected GCH1 and AADC homologs for enzyme kinetic studies, currently underway.

Conclusions: Molecular evolution studies of characteristic animal proteins reveal surprisingly close relationships between cyanobacterial and animal homologs, consistent with observations from other laboratories [4] and with proposals from ourselves [3] and others [5] that cyanobacteria have contributed to the evolution of animals. Experiment supports homology of biochemical functions. The biological functions of these proteins in cyanobacteria are largely unstudied.

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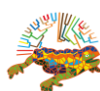
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Diverse origins of the last eukaryotic common ancestor proteins points to multiple interactions during eukaryogenesis

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POSTER SESSION 3 - P03.242, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The origin of the eukaryotic cell is one of the main evolutionary transitions. It is now broadly accepted that its complexity evolved from the interaction of at least two prokaryotic organisms, an alphaproteobacterium and Asgard archaeon. Nevertheless, whether other prokaryotic partners interacted in this transition remains under debate. To address this question, we reconstructed the proteome of the last eukaryotic common ancestor (LECA) and traced the origins of each protein family using phylogenomics and comprehensive prokaryote and viral protein datasets. We found evidence for

waves of gene transfers from at least three different bacteria, which are inferred to predate the acquisition of genes from the alphaproteobacterial ancestor of mitochondria. We further detected a non-negligible proportion of LECA gene families whose phylogenies suggest virus mediated gene transfers from prokaryotes, with Nucleocytoviricota being the most frequent transfer vector. Altogether, these results suggest that eukaryotes emerged from within a rich ecosystem where diverse biological entities interacted over prolonged periods of time.



Inferring the ancestral set of eukaryotic genes - comparative genomics of jakobid and malawimonad flagellates

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POSTER SESSION 3 - **P03.246**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background. Modern eukaryotes evolved from ancestral unicellular flagellates that already possessed hallmark features of their modern counterparts such as a nucleus, flagellum, endomembrane system, and mitochondria. Yet, many aspects of early eukaryotic evolution remain controversial, including the gene content of the ancestral mitochondrial eukaryote and the origin of canonical eukaryotic traits. To address these questions, we study jakobids and the distantly related malawimonads, two groups of bacterivorous unicellular eukaryotes often designated as 'primitive' due to their simple lifestyles and conserved ultrastructure. We hypothesize that they retain ancestral elements of the eukaryotic gene set.

Method. Using long-read nanopore data, we have assembled complete, chromosome-level genomes from five distant jakobids (including the anaerobic *Stygiella incarcerata*) and three malawimonads. We then identified proteins and ncRNAs genes, inferred gene models and annotated gene functions, to be used for comparative genome analyses.

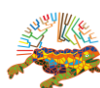
Results and Discussion. The genome architecture of these species is typically eukaryotic, with notable exceptions in *A. godoyi*, as described below. The genome assemblies enabled the identification of both haploid and diploid species. Diploids exhibit substantial sequence variation between haplotypes. All species encode the full set of core meiotic genes, supporting the hypothesis that meiosis predates the last eukaryotic common ancestor (LECA).

Chromosomes are generally linear, with telomeric repeats at both ends and short repeat motifs common across eukaryotes. A significant exception is *A. godoyi*, which possesses 60 linear and four circular nuclear chromosomes. These circular chromosomes encode genes involved in transcription, signal

transduction, metabolism, and transport, but lack core eukaryotic genes and show an unusually high proportion of genes encoding hypothetical proteins. Some genes on the circular chromosomes contain spliceosomal introns, supporting their eukaryotic origin and aligning with phylogenetic analyses. Intriguingly, the circular chromosomes also encode multiple copies of viral topoisomerase II and a recombinase, which likely play roles in maintaining chromosome structure during replication. The unusual gene composition of these chromosomes may represent either a relic of early eukaryotic evolution, possibly influenced by large viruses, or a lineage-specific curiosity of *A. godoyi*.

At the level of core gene functions, malawimonads have larger gene sets than jakobids. Genes are intron-rich in both groups, except for *A. godoyi*. Our predictions of U RNAs reveal the presence of both major and minor spliceosomes recognizing GU-AG and AT-AC splice sites in most species. However, *A. godoyi* and *Gefionella okellyi* possess only the major spliceosomal U RNAs and corresponding splice junctions. Splice junction conservation varies among species, extending to larger motifs than those found in fungi, animals, or plants. In one jakobid species, the 5' splice site motif resembles that of Group II introns.

To identify protein functional groups, we conducted HMM-based searches combined with subcellular targeting predictions (using three predictors, two of which are AI-based). Despite the lack of training data for "primitive" unicellular flagellates, the predictions appear robust. This supports the idea that mechanisms for protein targeting and subcellular localization were largely established before LECA, providing a framework for future comparative analyses of function and localization.



Orthology of eukaryotic protein complexes

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POSTER SESSION 3 - **P03.247**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Protein complexes are essential units of cellular life. Eukaryote genomic diversity is largely reflected in the repertoires of protein complexes encoded. Proteomic analyses that confirm protein complex formation are typically restricted to animals, plants and fungi, overlooking the majority of eukaryote genome variation that resides in protist genomes. However, there are multiple examples of pathways and constituent protein complexes, present in reference eukaryote genomes, that are also found in protists and therefore predicted to be conserved in the last eukaryotic common ancestor (LECA). To predict which protein complexes are conserved throughout eukaryotes and to what extent, we searched OrthoDB for genes corresponding both to proteins in the EBI Complex Portal and genomes from a subset of eukaryotes,

comprising an appropriate number of protists, animals, fungi and plants. We found that a minority of orthologous groups corresponded to protein complexes. However, those that did (compOGs) were more likely to be conserved in all eukaryote genomes queried than other orthologous groups. A spectrum of gene-duplication was observed within compOGs that were present in all queried species, but a general lack of expansion of genes related to DNA-repair was evident. Notably, a subset of compOGs was identified that included universally-conserved, minimally-expanded proteins from six protein complexes. These results are concomitant with the consensus view of a sophisticated LECA complexome, encoding multiple protein complexes, comprising single- and multi-copy proteins.



Pluralistic Red Queen and Court Jester Interactions Drive Unisexual Reproduction in Digital Populations

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POSTER SESSION 3 - P03.245, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

What drove the maintenance of sexual reproduction in early eukaryotes - and what drives it today - is a key question in evolutionary biology. Modelling and empirical studies that investigate this seemingly disadvantageous reproductive method are widespread. To date, there are no definitive answers regarding the ecological conditions or evolutionary benefits that may have led to the retention of sexual breeding. Unisexuality (mating without sexes or types) is a potential ancestral state of eukaryotic sex. This, and associated traits, are yet to be investigated in conjunction with abiotic forcing such as environmental change. Here, we use the eco-evolutionary model REvoSim to assess how biotic Red Queen, abiotic Court Jester,

and pluralistic drivers impact the prevalence of sex in a unisexual digital ecosystem over macroevolutionary timescales. Our results indicate that frequent antagonistic biotic interactions (pathogens/predators) and high rates of abiotic change both elicit a high proclivity towards sex in unisexual evolutionary agents. Pluralistic investigations demonstrate that a synergistic relationship exists between Red Queen and Court Jester drivers: a combination drives increased investment in sex. Abandon-Ship mechanisms allow sexual life cycles to break down maladapted epistatic relationships in stressful conditions, regardless of the ecological origin of that stress. We suggest that both biogenic and non-biogenic stressors could have maintained unisexuality in Proterozoic environments.



Modeling endosymbiotic gene flow at the origin of eukaryotes: a highways approach

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MEETING ROOM 129+130, August 21, 2025, 3:30 PM - 5:00 PM

The origin of the eukaryotic cell is considered one of the most enigmatic events in cellular evolution. The consensus view of eukaryogenesis centers on an endosymbiotic merger between two prokaryotes: an asgardarchaeal host with an alphaproteobacterial symbiont, the latter of which became the mitochondrion. However, debate remains over the sequence of events during eukaryogenesis and the possibility that other prokaryotes made genetic contributions to the early eukaryotic lineage, which may underlie specific cellular features. Since eukaryotes contain genetic contributions from their prokaryotic ancestors, phylogenetic approaches that account for the evolutionary history of eukaryotic genes of prokaryotic origin are especially useful. Phylogenies where eukaryotic genes nest within a donor prokaryotic clade provide context for gene acquisition into the nascent eukaryotic lineage. Despite this, it is challenging to ascertain the true extent of gene flow into the eukaryotic stem, in part due to the overwriting of the original genetic signal over the approximately two billion years of genome evolution that followed the

emergence of the first eukaryotes. To overcome this, we developed a new phylogenetic reconciliation approach that models “highways” of gene flow along the tree of life that may represent endosymbioses or long-term ecological associations and thereby can provide insight into prokaryotic genetic contributions along the eukaryotic stem. Our reconciliation method recovered known endosymbiotic associations, such as the prokaryotic contributions from the Alphaproteobacteria and the Cyanobacteria, ancestors of the mitochondria and plastid, respectively, in addition to the secondary transfer of higher-order plastids across the eukaryotic tree. Furthermore, we detected additional evidence of gene flow into the eukaryotic stem on highways from Chlamydiota, Plantomycetota, and Myxococcota. These findings suggest that in addition to the host and endosymbiont, a number of other prokaryotic players contributed genes into the emerging eukaryotic lineage, which could reflect the environmental and ecological conditions during eukaryogenesis.



Tracing the Prokaryotic Origins of Membrane Trafficking in Eukaryotes

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MEETING ROOM 129+130, August 21, 2025, 3:30 PM - 5:00 PM

The rise of eukaryotes is marked by the evolution of complex cellular architectures with endomembrane systems and intracellular trafficking. The Commander complex is critical for endosomal recycling and comprises ten COMMD proteins that form a heterodecameric ring. Although the COMMD proteins are conserved across eukaryotes, their evolutionary history remains elusive.

We used structure-based homology searches combined with genome-wide analyses to uncover deep prokaryotic homologues of the COMMD family. Despite minimal sequence similarity, bacterial and archaeal COMMD-like proteins conserve a distinctive domain architecture, featuring an N-terminal helical region and a C-terminal COMM domain. Biophysical analyses, crystallography, and cryo-electron

microscopy reveal that the prokaryotic proteins assemble into ring-shaped oligomers similar to the eukaryotic Commander core.

Phylogenetic analyses based on amino acid sequences and the 3Di structural alphabet show that the eukaryotic COMMD family is most closely related to the Myxococcota COMMD-like proteins. Our findings suggest that essential components of the eukaryotic membrane trafficking machinery were assembled from pre-existing bacterial systems through gene duplication, horizontal transfer, and structural innovation. These results highlight the contribution of ancient prokaryotic systems to the evolution of eukaryotes and offer new insights into the mosaic nature of eukaryogenesis.





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A comparison of selection on autosomes and the young X chromosome in *Mercurialis annua*

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MEETING ROOM 131, August 19, 2025, 2:00 PM - 5:00 PM

The evolutionary dynamics of proto-X and Y chromosomes during the early stages of their divergence - when most genes on both chromosomes remain functional, but recombination between them is suppressed - have been explored theoretically. A recent model predicts that genes on young X chromosomes should exhibit lower adaptive substitution rates than autosomal genes due to their smaller effective population size and the 'masking' of their gene products by their Y-linked allelic homologs in males, while they remain functional. Accordingly, young X-linked genes should tend to accumulate deleterious mutations faster than autosomal genes. This 'slower X effect' should be pronounced for male-biased genes on the X chromosome due to their differentially high expression in males. Here, we evaluate these predictions for sex chromosomes of the dioecious plant *Mercurialis annua*, whose Y

has a large non-recombining region but with its genes almost all functionally intact. We found that although autosomal and young X-linked genes are similarly divergent between *M. annua* and its sister species *M. huetii*, the X-linked genes segregate a higher proportion of non-synonymous polymorphisms. This observation points to the possible accumulation of deleterious mutations, consistent with the smaller effective population size of the X (as indicated by its lower diversity at silent sites). Tests of selection further indicate that the young X chromosomes indeed have a lower adaptive potential than the autosomes. The distribution of fitness effects for the X-linked genes was skewed towards deleterious mutations and devoid of beneficial. Taken together, these results are consistent with predictions of the slow-X effect.



A comprehensive phylogenomic view of the living xenarthran radiation

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MEETING ROOM 117, August 21, 2025, 2:00 PM - 5:00 PM

Xenarthrans (armadillos, anteaters, and sloths) represent one of the four main clades of placental mammals but remain underrepresented in genomic studies. Recent molecular evidence revealed hidden species diversity which suggests that additional species remain to be discovered. To address this knowledge gap, we generated 72 new xenarthran genomes constituting the most comprehensive genomic dataset for the group to date. By first analyzing 261 mitochondrial genomes, including several new museum specimens of three-toed sloths (*Bradypus* spp.) and pygmy anteaters (*Cyclopes* spp.), we confirmed divergent lineages and revealed potentially overlooked species diversity. Genome-wide comparisons of 92 individuals largely validated these mitogenomic findings, uncovering distinct species within the broadly distributed brown-throated three-toed sloth (*B. variegatus*), now restricted to the Amazon Basin, with *B. ephippiger* west of

the Andes and *B. griseus* in Central America. Similarly, we corroborated recently described pygmy anteater species and suggested two distinct lineages within *C. didactylus*. In armadillos, genome-scale data affirmed the four recently described *Dasypus* species and supported reclassifying all Euphractinae members under the genus *Euphractus*. Our phylogenomic analyses detected signals of incomplete lineage sorting and gene flow pointing to a few remaining phylogenetic uncertainties. Demographic reconstructions revealed a general decline in effective population sizes, with four species showing critically low genomic diversity, raising potential conservation concerns. Our study provides the first exhaustive assessment of the evolutionary history of xenarthrans at the genome level and identifies directions for future taxonomic investigations and conservation status assessments in this major placental clade.



A pluralistic approach to sex: a combination of coevolving parasite and increased mutation rate maintain outcrossing

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MEETING ROOM 115, August 21, 2025, 2:00 PM - 5:00 PM

The pervasiveness of sex in nature is a long-standing evolutionary enigma. Theory predicts that when a female in an outcrossing population gains the ability to reproduce uniparentally, uniparental reproduction will “invade” the population and eventually displace the outcrossing population due to the costs of sex. The Red Queen hypothesis, or the maintenance of outcrossing due to antagonistic coevolution, is predicted to maintain outcrossing when the antagonist (such as a parasite) incurs sufficient cost to the host. Alternatively, mutation-based hypotheses, such as the mutational deterministic hypothesis or Muller’s ratchet, are predicted to maintain outcrossing when mutation rates are sufficiently high. Given that highly virulent coevolving parasites and high mutation rates are not common in nature, neither hypothesis has been able to broadly explain the widespread distribution of outcrossing. The idea that multiple factors may act together to favor outcrossing, or the “pluralistic approach,” has been popular for decades, but very few empirical tests have been done. Here I provide an empirical test of the pluralistic approach by presenting the results of three evolution experiments using the mixed-mating nematode *Caenorhabditis elegans*. Each evolution experiment uses a previously established “selfing invasion” design: a small number of *C. elegans* capable of self-fertilization are introduced to an obligately outcrossing population with a shared genetic background, and selfing rates are tracked. The first two evolution experiments address the parameter space of the Red Queen hypothesis and mutation rate hypotheses, respectively. In the first experiment, nematode populations were coevolved with one of four strains of differently

virulent strains of the bacterial parasite *Serratia marcescens* in order to test the assumption that only high-virulent parasites favor outcrossing. The two most virulent strains were able to maintain outcrossing, while the low-virulent strains allowed a selfing invasion. In the second experiment, in order to test the hypothesis that high mutation rates can favor outcrossing, the mutation rate of *C. elegans* was artificially increased across a gradient from 1x to 8x the baseline mutation rate via chemical mutagenesis. The 4x and 8x treatments completely maintained outcrossing, while lower mutation rates allowed the selfing invasion. These two experiments together support the assumptions that both hypotheses only function under a specific parameter space and establish that parameter space within this system. The final evolution experiment tests the pluralistic approach by testing if some combination of low-virulent coevolving parasite and low mutation rate, which could not prevent the selfing invasion on their own, can widen this parameter space. The parasite strains and mutation rate treatments that were unable to impede the selfing invasion when acting alone were combined in a factorial design. Again we saw selfing invade the parasite-only and mutation-only treatments. However, outcrossing was maintained in some treatments where populations were exposed to both coevolving parasites and increased mutation, and invasion rate was slowed in other parasite-mutation combinations. These experiments provide much-needed empirical evidence for the pluralistic approach to outcrossing and open up the potential parameter space for both the Red Queen hypothesis and mutation-based hypotheses.



A proposal on how to study the multifarious environmental causes of natural selection

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The classic Lande & Arnold multiple regression approach allows us to better understand natural selection by decomposing fitness variation into contributions by various phenotypic traits. However, this only informs us on the organismal causes of fitness variation, but tells us nothing about the environmental causes of fitness variation. We suggest that environmental causes of natural selection can be estimated by also including environmental variables into the multiple regression. For example, bill size in a population of Darwin's finches might be found to be statistically related to reproductive success. But why? If we suspect that seed size has anything to do with it, we can include individual measures of available seed size (which may vary in space or in time) in our statistical model. Even more, selection on bill size might be hypothesised to depend on seed size. This can be tested by including the interaction term bill size * seed size into the model. Or perhaps it is hypothesised that larger bills prevent overheating of the finches. In that case we could include the interaction between individual bill size and the maximum temperature an individual is exposed to in the model, and see how much variance in reproductive success it explains. By including different environmental variables in the statistical model, we can estimate their apparent relative contributions to fitness. This seems relevant,

because natural environments are typically multidimensional, which means that selection on phenotypic traits would be expected to be multifarious too. However, the multidimensional nature of the environmental causes of selection is seldom acknowledged, let alone quantified. Our proposed approach allows for the multidimensionality of the selective environments to be quantified using variance partitioning and principal component techniques, whereby the importance of each selective environmental factor is evaluated by quantifying how the interaction between the factor and the phenotypic trait of interest affects fitness or a fitness component. This approach can be further generalized to include both interactions between the selective environmental factor and the squared component of the trait (to evaluate stabilizing or disruptive selection) as well as interactions between different selective environmental factors (e. g. environmental variables E1 and E2), which would further reveal the multidimensionality and interrelatedness of the selective environment. Using this approach in combination with classic techniques for quantifying natural or sexual selection can increase our understanding of how the environment drives phenotypic selection in natural populations, and allows us to quantify the relative importance of different selective agents.



Adaptive capacity, climatic vulnerability and reintroduction success of small populations: insights from an Australian freshwater fish

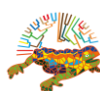
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MEETING ROOM 116, August 19, 2025, 11:00 AM - 1:00 PM

Understanding how biodiversity responds to rapid environmental change is a global research priority. This is key for identifying biodiversity vulnerability and resilience, and for guiding conservation planning now and in the future. Freshwater fishes are highly susceptible to decline following anthropogenic disturbance and climate extremes. Here I summarise results from a long-term conservation genomics programme of the southern pygmy perch (*Nannoperca australis*), a threatened freshwater fish from southeastern Australia. Being small and dispersal-limited, this is a surrogate indicator for many species that must adapt to climate change in situ to avoid extinction, and for species in need of conservation reintroductions and assisted gene flow. Our programme integrates information from genomics, gene expression, and modelling of genotype-environment associations and vulnerability

under present and future climates from across the range of the species. It also includes the longitudinal monitoring of genomic diversity of a conservation reintroduction. Most populations showed small effective size, low genetic diversity, pronounced inbreeding, and high isolation due to recent habitat fragmentation. There was genomic evidence of local adaptation to seasonal variation in temperature and rainfall, and of adaptive responses in gene expression due to climatic heterogeneity. Vulnerability to climate change correlated with elevation, with upland populations projected to be the most maladapted to future climates. Encouragingly, the adaptive potential of a locally extinct population was maintained via genetically informed captive breeding and reintroduction. These findings enhance our understanding about adaptive climatic capacity of small populations and exemplify benefits and challenges of applying genomics to conservation.



Adaptive evolution of viviparity resists introgression at genome regions housing genes functional for reproduction

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S51.10 - Sex, MEETING ROOM 115, August 21, 2025, 2:00 PM - 5:00 PM

Evolutionary innovations represent adaptive step-changes for biodiversity, but rarely are the genetic processes and pace of novelty demonstrated. Live-bearing young (viviparity) is a classic example of such a major evolutionary transition and has evolved from oviparity many times. In this study, we reconstructed the evolutionary molecular mechanisms at the origins of viviparity using a reproductively bimodal lizard species, the Eurasian common lizard. We whole genome sequenced 101 lizards from populations across the distribution, representing all phylogeographic lineages and mapped to a new reference genome. We found that the history and genomic architecture of viviparity is complex. Strong selection for viviparity is evident in regions

genome-wide. Demographic reconstructions showed that viviparity prompted diversification and population growth that is not mirrored in oviparous clades. Gene flow between parity modes was extensive and long-term but resisted at many genomic regions. These genomic barrier regions are enriched for genes we show are functional in pregnancy and reproduction, as inferred from natural populations and a common garden experiment on this species, and previous research on other amniotes. We found no evidence for a role of protein coding variations in the evolution of viviparity. Our study demonstrates that viviparity in an amniote evolved by the gradual accumulation of regulatory mechanisms genome-wide.



An overview of open science in eco-evo research and the publisher effect.

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MEETING ROOM 117, August 18, 2025, 2:00 PM - 5:00 PM

Open science is broadly recognized to be part of the solution to the reproducibility crisis affecting science. There is increasing incentive for scientists to publish in open access journals, share data and share code [1]. If today most of us would agree that transparency is good scientific practice, only 10 to 15 years ago it was rare for the data or code associated with a research study to be made available alongside with the publication. There is a need of monitoring the rise of open science in eco-evo, and identifying the factors that promote or hinder its development [2, 3]. Here we analyzed 550 articles published in 2024 in 110 eco-evo journals randomly sampled from the DAFNEE database [4]. We estimated the rate of data and code sharing and related it to journal subfield, business model, partnership, and publisher. We provide an

overview of the current state of open science in eco-evo, which will serve as a milestone in monitoring this move. Importantly, we uncover a strong publisher effect, and report that publishers who partner with academic institutions are the ones who tend to promote open science.

[1] Poisot et al. 2019. Ecological data should not be so hard to find and reuse. *Trends in Ecology and Evolution* 6:494-496.

[2] Culina et al. 2020. Low availability of code in ecology: A call for urgent action. *PLoS Biology* 18:e3000763.

[3] Ivimey-Cook et al. 2025. From policy to practice: progress towards data- and code-sharing in ecology and evolution. *EcoEvoRxiv* <https://doi.org/10.32942/X2492Q>

[4] DAFNEE, a database of academia-friendly journals in ecology and evolutionary biology. <https://dafnee.isem-evolution.fr>



Antibiotics of the future induce the evolution of hypervirulent bacterial pathogens

Pál C¹

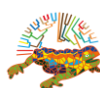
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MEETING ROOM 116, August 19, 2025, 11:00 AM - 1:00 PM

Several new antibiotics are in the development pipeline, but it's uncertain whether selection for resistance unintentionally results in more virulent strains during treatment. Here, we study the consequences of resistance evolution on virulence in four Gram-negative bacterial pathogens, focusing on 20 antibiotics that have not yet reached the market. Using multiple infection models, we found that several antibiotics with different modes of action induce the evolution of hypervirulent bacteria, which is a cause for concern. These

bacteria simultaneously displayed an enhanced ability to invade human epithelial cells and to evade the human innate immune system. The mutations underlying these changes are already present in natural bacterial populations, often altering lipopolysaccharide production and thereby causing structural changes in the bacterial cell envelope. Our work may guide the safe clinical use of new antibiotics.

See also: Daruka et al. Nature Microbiology 2025



Are long-lived species doomed to slow adaptive evolution?

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MEETING ROOM 116, August 21, 2025, 2:00 PM - 5:00 PM

Conventional wisdom suggests that adaptive evolution should occur more slowly in long-lived organisms than in short-lived ones due to longer generation times. As a result, long-lived organisms are often viewed as less capable of responding to rapid environmental change. However, empirical evidence supporting this assumption is lacking. Comparative studies across taxa show no consistent relationship between generation time and rates of adaptive change. Moreover, molecular data reveal that adaptive substitution rates can even correlate positively with longevity in some animal groups, and high rates of molecular adaptation have been documented in long-lived plants and vertebrates. The misconception likely arises from conflating molecular evolution, measured as neutral substitution rates, with adaptive evolution. While neutral mutation rates tend to decline with increasing generation time, due to fewer DNA replications per unit time, this does not necessarily translate into slower adaptive evolution. This is because adaptation depends not only on mutation but also on natural selection and other evolutionary forces. A fundamental flaw in the assumption is the idea that evolution occurs across generations. This idea overlooks the fact that differential survival can causally contribute to adaptive allele frequency changes within a single generation, providing a pathway for rapid adaptation. In long-lived

species, this selection via survival may be especially potent. I introduce the concept of the "filtering effect" to describe how survival-based selection can be amplified by longevity. Specifically, when survival influences fitness over long pre-reproductive periods, even small differences in intrinsic mortality can generate substantial selective advantages. The filtering effect thus scales non-linearly with lifespan, enhancing the efficiency of selection in long-lived organisms. To explore these ideas, I present a theoretical model that examines how longevity influences the rate of adaptation under environmental change. The model incorporates realistic life history traits of long-lived species, such as delayed reproduction. It reveals that when fitness is determined solely by fecundity, the conventional view holds: short-lived species adapt more rapidly, as evolution proceeds only across generations. However, when survival contributes to fitness, the rate of adaptive evolution becomes independent of longevity. These findings challenge the prevailing view that long-lived species are inherently slow to adapt. A paradigm shift is thus needed, one in which long-lived organisms are not necessarily doomed to slow adaptive evolution. This new paradigm has important implications for evolutionary theory, conservation biology, and our understanding of species' responses to global environmental change.



Asymmetry in vertebrate gene repertoires: what happened to the shark HoxC cluster?

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Research efforts have long focused on the immediate consequences of whole genome duplications (WGDs), but their evolutionary impacts may persist far longer than previously appreciated. In fact, some vertebrate genes that survived the post-WGD period were lost independently in multiple lineages hundreds of millions of years later. For instance, while no genomic loss of Pax6—a key regulator of the central nervous system—has ever been documented, its sister gene Pax4 is absent in several vertebrate classes, despite its role in islet cell differentiation in mammals. Exhaustive searches for such 'elusive' genes have revealed systematic patterns in their sequence features and particular dispositions toward transcription, replication, and epigenomic regulation (Hara & Kuraku, *eLife* 2023, 12:e82290). Importantly, these properties extend beyond the coding regions into the flanking genomic contexts (Hara & Kuraku, *Trends Genet*, accepted). We are now extending this concept to a vertebrate-wide perspective through our work with the Squalomix consortium (<https://squalomix.github.io>), a collaborative initiative advancing omics-

driven biology of sharks and rays. As one of the most ancient vertebrate lineages, sharks and rays offer unparalleled opportunities for evolutionary genomics owing to their large genomes and distinct karyotypes (Uno et al., *Commun. Biol.* 2020, 3:652; reviewed in Kuraku, *Dev. Biol.* 2021, 477:262–272). A particularly intriguing discovery from our shark studies is the presence of HoxC genes (Hara et al., *Nat. Ecol. Evol.* 2018, 2:1761–1771; Yamaguchi et al., *Genome Res.* 2023, 33:1527–1540)—previously thought to be absent in this group. These genes exhibit highly divergent sequences, yet maintain embryonic expression patterns consistent with the canonical 'Hox code.' Remarkably, the HoxC clusters in sharks and rays are heavily invaded by repetitive elements, leading to substantial cluster elongation. In contrast, the HoxA, -B, and -D clusters have remained compact—around 100 kb—even in these enlarged genomes. These findings shed new light on how the fates of regulatory genes are shaped by intragenomic heterogeneity and suggest that non-genic genomic features play a crucial role in long-term gene retention and function across vertebrate evolution.



Breakdown of speciation processes between two genetically and ecologically divergent ecotypes of a wood decay fungus

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S51-01 - Ecology, MEETING ROOM 117, August 18, 2025, 10:30 AM - 12:30 PM

Speciation is often portrayed as a simple bifurcating process, but it frequently involves more complexity, including gene flow between diverging lineages. Here, we present a study of the wood decay fungus *Meruliopsis taxicola*. We document ongoing hybridization and potential breakdown of speciation processes between two ecologically and genetically divergent lineages. We sequenced genomes of samples collected throughout the distribution of *M. taxicola* in Fennoscandia. Our genomic analyses reveal strong divergence between two ecotypes: one predominately associated with spruce (*Picea abies*) in more continental areas (the spruce ecotype) and the other mainly on pine (*Pinus sylvestris*) in coastal areas (the pine ecotype). Notably, we identified several genomic intermediates forming a hybrid zone between these ranges. Distribution modeling supports that the ecotypes occupy different climatic niches, forming a hybrid zone in the overlapping distributions. The pine ecotype exhibits inbreeding and reduced genetic diversity compared to the spruce ecotype, while hybrids display intermediate diversity.

Demographic analysis suggests that the ecotypes were panmictic prior to the last glacial period and likely diverged in separate glacial refugia. As the ice retreated, the ecotypes migrated together with their respective host species into Fennoscandia, resulting in secondary contact. To assess potential differential ecological adaptations, we conducted growth experiments under varied temperatures and water potentials. The spruce ecotype generally exhibited faster growth rates in all treatments than the pine ecotype, with hybrids showing intermediate growth rates. Our findings suggest an ongoing speciation process, with two ecotypes adapted to different substrates and climates, where differences in growth rates reflect adaptations to different habitats. Hybridization between the ecotypes may lead to a potential reversal of speciation. Alternatively, a stable hybrid zone between the distributions of the ecotypes has established a third admixed ecotype.



Can sexually selected traits influence the efficacy genetic rescue?

An evolve and re-sequence approach

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MEETING ROOM 115, August 21, 2025, 2:00 PM - 5:00 PM

Habitat fragmentation and population isolation are becoming increasingly common across our changing world, elevating the risk of inbreeding, loss of genetic diversity and adaptive capacity, ultimately, increasing localised extinction risk. Genetic rescue - where population fitness improves following the introduction of novel genetic variants via translocated individuals - is a promising conservation strategy for mitigating these risks. However, there are associated risks, such as the introduction of masked genetic load. Understanding factors that can optimise genetic rescue success is therefore a key priority. One such factor may be the genetic quality of the introduced individuals, which can be reflected in their investment of costly sexually selected traits. These traits may influence the reproductive success of rescuers, introgression rates and amount of introduced masked load. Despite their potential importance, the role of sexually selected traits has rarely been considered in the context of genetic rescue.

We used an evolve-and-resequence approach in the bulb mite (*Rhizoglyphus robini*), which exhibits discrete male morphs, to test whether expression of a condition-dependent sexually selected trait affects the efficacy of genetic rescue in inbred populations. We tracked reproductive potential across generations following the introduction of males with or without a costly sexually selected trait and subsequently assessed population resilience under thermal stress. Our results demonstrate that genetic rescue can enhance population fitness, with these effects persisting across multiple generations. However, the timing of benefits varied: males lacking the sexually selected trait conferred more immediate improvements, while those expressing the trait had more pronounced long-term positive

effects. Additionally, we show genetic rescue generally reduced extinction risk under environmental stress. Despite broadly positive effects of genetic rescue, a proportion of genetic rescue attempts also failed, indicating some idiosyncratic effects likely exist.

Using pool-sequencing, we also quantified genome-wide diversity before and after rescue, estimated the number of fixed, presumed deleterious mutations in inbred populations, and tracked the introgression of novel and potentially deleterious variants. We found that populations with the lowest initial genetic diversity and fitness benefited most from rescue. When successful, rescue introduced new variants that likely masked fixed deleterious variants from inbred populations, and although rescuers often introduced more masked load than the fixed presumed deleterious variants in inbred populations, the net effect on population fitness was positive. Whether rescuers expressed sexually selected traits or not had no consistent influence on differences in changes of genome-wide diversity or quantity of introduced deleterious mutations. However, as introduced load increases in frequency and its effects exposed, coupled with sexually selected traits shaping the timing of fitness benefits, suggests that on average males expressing sexually selected traits carry weaker, though not fewer, deleterious mutations.

Overall, our findings highlight that genetic rescue can yield substantial, lasting benefits even when masked load is introduced. Moreover, condition-dependent sexually selected traits may shape the timing of these benefits. These insights can help inform design and implementation for more effective genetic rescue strategies in conservation programmes.



Can transmissible tumors manipulate their hosts ?

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While host phenotypic manipulation by parasites is a widespread phenomenon, whether tumors – which can be likened to parasite entities, can also manipulate their hosts is not known. Theory predicts that this should nevertheless be the case, especially when tumors (neoplasms) are transmissible. We explored this hypothesis in a cnidarian Hydra model system, in which spontaneous tumors can occur in the lab, and lineages in which such neoplastic cells are vertically transmitted (through host budding) have been maintained for over 15 years. Remarkably, the hydras with long-term transmissible tumors show an unexpected increase in the number of their tentacles,

allowing for the possibility that these neoplastic cells can manipulate the host. By experimentally transplanting healthy as well as neoplastic tissues derived from both recent and long-term transmissible tumors, we found that only the long-term transmissible tumors were able to trigger the growth of additional tentacles. Also, supernumerary tentacles, by permitting higher foraging efficiency for the host, were associated with an increased budding rate, thereby favoring the vertical transmission of tumors. To our knowledge, this is the first evidence that, like true parasites, transmissible tumors can evolve strategies to manipulate the phenotype of their host.



Casting light on the role of light-sensitive bacteria in plant adaptation to polar light regimes

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MEETING ROOM 117, August 18, 2025, 10:30 AM - 12:30 PM

Rapid climate warming expands ice-free habitats in polar regions, enabling plants to track climate change by migrating poleward. To establish in this novel habitat space, however, the species must adapt to higher latitudes' more extreme climate conditions. In polar regions, plant growth is particularly limited by seasonal variation in day length: above the polar circles, plants must cope with periods of polar day and polar night. Thus, poleward-dispersing plants must adapt their phenology to the more rapid advancement of spring and the brief but intense growing season. However, plant-associated microbes also play a notable role in plant adaptations to physiological stressors caused by climate variation. The growth of microbes is limited by climate factors and the availability of suitable hosts, but their dispersal ability is multitudes greater than that of plants. As such, microbes likely impact the success of plant colonization across polar regions through host-dependent and climate-driven interactions.

In our studies, we have investigated the distribution and community composition of a specific group of light-sensitive bacteria, AAPB (Aerobic Anoxygenic Phototrophic Bacteria), in the endo- and phyllospheres of polar plant species. Having been recently discovered in terrestrial systems, the biogeographic patterns and host-dependent interactions of AAPBs remain largely unknown. However, previous studies suggest AAPBs to be near-ubiquitous in the tissues of numerous plant species across different terrestrial environments, including high latitudes and elevations. Considering this frequent association, we hypothesize that their light-harvesting machinery, unlike that of plants, may play a role in plant adaptation to different light regimes. Such an adaptive relationship would be particularly advantageous in polar regions characterized by great seasonal variation in light availability.

To unravel potential distribution patterns of AAPBs, we present the first screening of AAPBs associated with numerous polar plant species across a range of latitudes around both hemispheres: Greenland, Svalbard, Northern Finland, Chilean Patagonia, and maritime Antarctica. We found AAPBs across all the sampled plant tissues, most commonly in phyllospheres but also in leaf and root endospheres. The most abundant AAPB genus identified was *Sphingomonas*, frequently found in all geographic regions. In contrast, we found the proportion of *Methylobacterium* to decrease from one of the biggest groups at sub-arctic and sub-antarctic sites to singular observations or absent at higher latitudes. This suggests a taxonomic turnover, which may be linked to host-dependent interactions, poleward-declining plant diversity, or milder growth conditions at lower latitudes.

Following the results of the previous study, we will conduct a mesocosm experiment to take the first step toward disentangling the potentially adaptive relationship between AAPBs and their plant hosts. In this experiment, we will inoculate one of AAPB's plant hosts, *Deschampsia antarctica*, with a selection of AAPB strains. The effects of AAPB addition will be measured through plant performance in growth chambers under distinct light regimes, simulating high and low latitude light environments. As *D. antarctica* is currently undergoing a poleward range expansion in maritime Antarctica, focusing on the species provides a unique opportunity to study the effects of AAPBs on plant adaptation to more extreme light environments. We predict that the increased availability of AAPBs will affect host adaptation to different light conditions. Together, our results call attention to the light-sensitive microbiome in projecting plant adaptation to changes in seasonal light availability, opening a new avenue of research into plant-microbe interactions.



Comparative analysis of predator-induced plastic responses in tadpoles and its regulation by corticosterone

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Organisms are often exposed to great spatial and temporal environmental heterogeneity. Consequently, most organisms have evolved the ability to perceive such fluctuations and adjust their phenotype accordingly. Environmentally-induced responses may be quite complex, and require modifications in their physiology, behaviour, morphology and even life histories. Such phenotypic alterations are usually driven by hormonal shifts that result in differential gene expression across tissues. A paradigmatic example of adaptive plasticity are predator-induced defences displayed by preys upon detection of predators. Amphibian larvae are generally capable of perceiving predators either chemically or visually, and can often alter their behaviour and morphology in a way that reduces their risk of predation, although the extent of the plastic shifts induced seems to vary considerably among species. Inducible defences in amphibian larvae are thought to be regulated by the hypothalamus – pituitary – interrenal (HPI) axis. However, previous studies have reported conflicting results regarding the role of glucocorticoids (corticosterone, CORT) in regulating these responses in amphibian larvae. Here we take an experimental

comparative approach to study the role of corticosterone in the inducible responses of tadpoles to natural predators, across twelve anuran genera. By experimentally exposing larvae from each species to the presence / absence of predator cues, or to different levels of exogenous corticosterone, we evaluated the effect of these two strong inducers on activity rate, morphology and life history traits. In addition, we measured the endogenous corticosterone concentration and oxidative stress level of our experimental tadpoles. We observed extensive interspecific variation in their degree of plasticity and the responsiveness of CORT expression. The morphological and behavioural responses to predators across species were generally decoupled from each other and dissimilar to their responses to increased corticosterone. Despite the limited number of species compared in this study, we present solid evidence suggesting that tadpoles' defences against predators can be controlled by a distinct physiological mechanism than CORT, and that additional aspects of amphibian development, such as developmental rate, may be relevant in understanding variation in predator-induced responses.



Comparative Population Genomics Illuminates Species Boundaries and Symbiotic Disruption in *Eunicella* Octocorals

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MEETING ROOM 116, August 21, 2025, 2:00 PM - 5:00 PM

The process of speciation is a central topic in evolutionary biology due to its role in shaping biodiversity patterns. We investigate species boundaries in three accepted octocorals: *Eunicella verrucosa* (Pallas, 1766), *E. cavolini* (Koch, 1887), and *E. singularis* (Esper, 1791). *E. verrucosa* is widely distributed in the Eastern Atlantic and Mediterranean Sea while *E. cavolini* and *E. singularis* are only found in the Mediterranean. *E. singularis* hosts a photosynthetic *Philozoon* species contrary to the two other species.

Using whole genome re-sequencing data from 20 individuals from 13 localities, we i) characterized the genetic relationships between individuals of the three species; ii) looked for the genomic landscapes of differentiation; and iii) provided first insights into the associated microbiome using a metagenomics framework.

Analyses of genome-wide diversity and divergence reveal clear genetic differentiation among the three recognized species; *E. verrucosa* being the most divergent one. However, a triangular hybrid plot based on ancestry-informative markers revealed two potential hybrids and one backcrossed individual between *E. cavolini* and *E. singularis*. Genome scan analyses did

not reveal distinct genomic islands of divergence between *E. cavolini* and *E. singularis* in line with a scenario of recent divergence with ongoing gene flow. Comparing the genomic landscape of the potential hybrids, we identified distinct genomic regions with ancestry from either *E. singularis* or *E. cavolini* supporting different hybridization events.

The metagenomic analysis focused on the occurrence of *Philozoon* supported the absence of the symbiont in the potential *E. cavolini* / *E. singularis* hybrids suggesting a disruption in the evolutionary stability of the symbiotic association following the hybridization.

This study provides an example of how gene flow and hybridization influence species boundaries and biodiversity patterns in octocorals. The observed hybridization raises important questions about the stability of symbiotic relationships in *E. singularis* and its effects on hybrid fitness and adaptability. Detailed characterization of hybrid zones and scenario-based demographic inferences will deepen our understanding on the evolution of octocorals. This knowledge is required to contribute to ongoing conservation and restoration efforts.



Complex genomic content allows for rapid giant virus adaptation to a novel host

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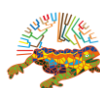
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Giant viruses contain genomes and particles with sizes and dimensions that rival those of bacteria. These viruses, which primarily infect protists, may have evolved through a long history of genome expansion and contraction, allowing them to adapt to changing environments and host pressures with flexibility. In this study, we experimentally explored how giant viruses evolve in response to novel host conditions by serially propagating three distinct viruses — Acanthamoeba polyphaga mimivirus, Marseillevirus sp. Vienna, and Tupanvirus deep ocean — in three different amoeba hosts.

Mimivirus and Marseillevirus were passaged in two strains of Acanthamoeba. While Mimivirus maintained stability, Marseillevirus exhibited a notable loss in infectivity and viral replication success throughout the evolution experiment, suggesting limits to its host range plasticity. In contrast, Tupanvirus, one of the largest and most complex viruses known to date, which is usually propagated in

Acanthamoeba, was also challenged to evolve in a host to which it is maladapted, Vermamoeba. Initially, in this “novel” host, viral replication success was low, with few genomic changes and minimal cytopathic effects, indicating a strong bottleneck effect. Remarkably, after only seven weeks of evolution, Tupanvirus had evolved to significantly increase its replication success and host impact in Vermamoeba, producing approximately eight times more viral progeny than the ancestral strain. These changes likely reflect the evolution of mechanisms to manipulate host cell behaviour, facilitating more efficient viral propagation.

Our findings support the notion that giant viruses can rapidly adapt to novel hosts, possibly through the genomic flexibility afforded by their large and complex genomes. This adaptability may offer a competitive advantage in environments with low host availability, where every host encounter counts, further explaining the evolutionary pressures that favour genome expansion in giant viruses



Condition matters: First insights into alarm cue production in a freshwater gastropod.

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Predation is one of the most pervasive selection pressures faced by prey individuals over the course of their lives. Thus, the ability to detect and avoid predation threats is critical to individuals' lifetime fitness. Freshwater snails, such as the classic plasticity model system *Physella acuta*, use chemical alarm cues released by injured conspecifics as a source of information regarding local predation risk. Recent studies show that donor condition impacts chemical alarm cue production, with high-condition donors producing cues that differ in composition and effectiveness at inducing antipredator behaviour compared to those from low-condition donors. Hence, prey organisms may respond differently to alarm cues depending on the donor's condition, with potential implications for their ability to estimate predation risk and induce defensive behaviours. In our study, we focus on the condition-dependence of alarm cues in *P. acuta* to address this issue and employ a combination of analytical and behavioral

trials. First, we extract alarm cues from high and low-condition donor snails. We then profiled the specific chemical compounds present in these cues using gas chromatography-mass spectrometry (GC-MS). Second, we tested the efficacy of these cues by studying crawl-out behaviour in *P. acuta* before and after exposure to high-condition alarm cues, low-condition alarm cues or a water control treatment. Here, we report our first results showing to what extent alarm cue production in *P. acuta* is condition-dependent. Additionally, we report our first insights into potential key compounds within *P. acuta* alarm cues. Furthermore, we may also be able to provide insight into the biological concentration levels at which these profiled compounds are found in *P. acuta*. Altogether, our study may provide valuable insights into the chemical communication system of gastropods as well as the mechanisms underlying their estimation of predation risk.



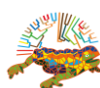
Continental-scale variation of *Saccharomyces paradoxus*

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MEETING ROOM 117, August 18, 2025, 10:30 AM - 12:30 PM

Saccharomyces paradoxus is the never-domesticated sister species of *Saccharomyces cerevisiae*. *S. paradoxus* represents an emerging model for investigating natural genomic diversification and adaptive variation in wild yeast populations. Here, through a network of 60 laboratories, we conducted a deep sampling campaign across temperate forests in Europe and the Mediterranean basin to reveal continental patterns of genomic variation. We collected over 5,000 environmental samples from oak trees across 150 sites and recorded geographical and ecological metadata. We frequently isolated *Saccharomyces paradoxus* with varying success rates and defined its geographical range. Its occurrence dominates from Eastern to Western Europe, with a gradual decline at lower latitudes. Genome sequencing of over 700 isolates shows patterns of genetic variation likely influenced by dispersal and climate zones.

We measured low levels of genetic variation, which mirror those of other wild *Saccharomyces* isolates, suggesting either a recent bottleneck or a founder event with subsequent demographic expansion across the continent during the post-glacial period. By scanning the genome, we identified a locus segregating at intermediate frequencies, perhaps maintained by balancing selection and contributing to antifungal resistance, and we confirmed the presence of a fixed *S. cerevisiae* introgression on chromosome XIV. Moreover, we discovered a novel, rare *S. paradoxus* lineage with large blocks of introgressed DNA from an unknown donor *Saccharomyces* population, influencing the rare lineage's ability to process galactose. This population genomic resource provides novel insights into *Saccharomyces paradoxus* at fine ecological and geographical scales.



Deleterious mutations cause evolution of lifespan extension by dietary restriction

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MEETING ROOM 116, August 21, 2025, 2:00 PM - 5:00 PM

Dietary restriction (DR) extends lifespan in animals and plants, but the evolutionary causes are elusive. Adaptive hypotheses posit that DR extends lifespan because resources are reallocated from reproduction to survival ('disposable soma'); models assume that organisms recycle cellular waste to maximize either their immediate reproduction ('nutrient recycling') or survival ('clean cupboards'). We developed an experimental paradigm that tricks *Caenorhabditis elegans* nematodes into increasing their reproductive effort under DR via food odour, thus allowing us to test these hypotheses. We found that experimentally increased reproduction under DR does not affect immediate or long-term survival benefits compared to DR animals that did not reproduce, thus refuting all three

adaptive hypotheses. Our data suggests that a large part of suppressed fertility under DR is a result of organisms refraining from producing offspring in a poor environment. We developed a model based on Hamiltonian forces of selection to show that lifespan extension under DR evolves because DR suppresses fertility, directly increasing selection against mortality in DR environment. Our analytical approach suggests that DR-driven lifespan extension can evolve under a broader range of conditions not previously anticipated, such as a relaxed need for physiological or genetic trade-offs or for individuals to experience multiple environments. We show that the evolution of DR is compatible with mutation accumulation models of senescence.



Dietary choice and life history in *D. melanogaster*

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MEETING ROOM 118+119, August 19, 2025, 2:00 PM - 5:00 PM

Diet quantity and quality is a fundamental determinant of organismal fitness. However there is significant variation across life stages and sexes in which diets are optimal to sustain high reproductive output. Studies in *Drosophila melanogaster* fruit flies show that diets that maximise reproduction may also shorten lifespan and the extent of such effects may also differ across the sexes. It is not yet clear how these multiple interacting pressures manifest in an individual's dietary choices throughout life. In particular, it is not known whether a female's oviposition site choice across her reproductive lifetime is consistently beneficial for offspring or indeed whether nutritional choices made by

larvae are in line with the oviposition site choices of their mothers. I tested these questions by using three-way dietary choice assays for solid diets varying in Protein: Carbohydrate ratios. This was then followed by no-choice assays of larval survival and developmental time, to assess the life history effects of the dietary choices made by females and larvae. The results showed an unexpected interaction of parental age with offspring diet in terms of offspring survival and allometry, with differing effects on male versus female offspring. More broadly, the work is important within the wider context of revealing fundamental determinants of sex- and-stage specific plasticity in life history.



Differences in responses to seminal fluid between European populations of the simultaneous hermaphrodite *Lymnaea stagnalis*

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MEETING ROOM 131, August 19, 2025, 2:00 PM - 5:00 PM

Individuals influence conspecifics through diverse interactions. Such positive and negative social effects caused by relatives, competitors or mating partners affect fitness. Well-studied interactions include visual, pheromonal and auditory communication, but senders can also transfer biological materials that intentionally bypass sensory organs and directly affect the physiology of the receiver. Examples of such socially transferred materials are found in milk, yolk, mucus, saliva and ejaculate. Focussing on the latter, we will show how freshwater snail donors transfer accessory gland proteins (ACPs) in their seminal fluid that affect the reproductive physiology of the recipient (i.e.

mating partner). To test whether the effectiveness of ACPs differs, we compared seven standardised European populations taking genomic distance into account. We focussed on the well-established effect of the ACP Ovipostatin (or LyAcp10), which has been shown to temporarily suppress egg laying and increase investment per egg. We tested for these effects using natural inseminations and intravaginal injection bioassays. For natural inseminations we also quantified mating behaviour. The population differences inform us about how conserved the responses to ACPs are and whether they can lead to reproductive isolation.



Disentangling plasticity from genetics: reproductive effort of urban and forest great tits in a common garden experiment

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MEETING ROOM 131, August 19, 2025, 2:00 PM - 5:00 PM

Phenotypic plasticity is widely recognized as a key mechanism through which organisms cope with rapid environmental change such as urbanisation. Understanding whether phenotypic shifts in urban populations are driven by plasticity or by genetic change is critical for predicting their adaptive potential. In birds, urban individuals often have earlier breeding phenology and smaller clutches than their forest-dwelling conspecifics—patterns generally attributed to elevated temperatures and reduced food availability in urban areas. However, it remains to be demonstrated whether earlier breeding in cities results from individual phenotypic plasticity or from population adaptation.

To disentangle genetic from environmental contributions to these differences in reproductive traits, we conducted a common garden experiment using great tits (*Parus major*) originating from unincubated eggs collected in urban and forest habitats. Chicks were hand-reared under identical environmental conditions. The following spring, birds were paired by habitat of origin and placed in breeding aviaries, where we recorded their laying date and clutch size.

Contrary to patterns observed in wild populations, differences in laying date linked

to habitat of origin disappeared under common garden conditions. While laying date was unaffected by the habitat of origin, it was significantly influenced by the type of aviary, with individuals laying earlier in aviaries that were more familiar to them—suggesting a strong influence of environmental familiarity or stress on reproductive timing. Interestingly, clutch size differences observed in the wild were reversed in the common garden, with females of urban-origin producing larger clutches than their forest counterparts.

These findings suggest that the observed variation in reproductive traits is primarily due to phenotypic plasticity rather than genetic divergence. When the strong environmental constraints of urbanisation were removed, urban great tits expressed clutch sizes—a heritable trait—in line with predictions of positive selection for larger clutches in urban environments. This pattern likely reflects a counter-gradient variation, where genetic and environmental effects act in opposing directions, resulting in cryptic microevolutionary change. Overall, our common garden study emphasises the need to consider phenotypic plasticity and environmental stress when evaluating adaptive responses in urban ecosystems.



Diversification of stickleback sex chromosomes driven by introgression

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MEETING ROOM 131, August 19, 2025, 2:00 PM - 5:00 PM

Recent studies on origin and processes driving sex chromosome evolution have highlighted introgression as a potential factor shaping sex chromosome evolution, including a study of one evolutionary lineage (EL) of nine-spined sticklebacks (*Pungitius pungitius*). We constructed haplotype-level assemblies for two additional *P. pungitius* lineages (WL and UK), as well as for their sister species, *Pungitius laevis*. Using whole genome resequencing data, we identified a short (~470 kb) sex determination region (SDR) in the WL Chr3, which shares the most recent common ancestor not with the X chromosome, but with the homologous

region in *P. laevis*. This suggests that SDR in WL has been subject to introgression from *P. laevis*. While the SDR in the UK lineage remains unknown, the region homologous to WL SDR in UK lineage is divergent from that in WL and also subject introgression from *P. laevis*. This may suggest that sex determination mechanism in UK lineage is different from that in WL and EL. Our results highlight the extreme intraspecific diversity of young sex chromosomes and sex determination systems in sticklebacks and indicate important role for introgression in shaping the diversification of sex chromosomes in stickleback fishes.



Domestic dog introgression in Australian dingoes: environmental drivers and evolutionary consequences

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MEETING ROOM 117, August 18, 2025, 10:30 AM - 12:30 PM

Hybridization between wild and domestic canids is a widespread phenomenon with significant implications for genetic diversity, local adaptation, and conservation management. In Australia, hybridization between dingoes and domestic dogs represents a dual conservation challenge: compromising the genetic integrity of dingoes while facilitating potential adaptive introgression. To explore the genetic and environmental drivers of hybridization and the evolutionary consequences of this process, we analyzed SNP data generated using a high-density microarray (SNP chip), including 336 dingoes and 396 European domestic dogs, encompassing 170,465 SNPs. Despite the genetic distinctiveness between dingoes and domestic dogs, hybridization occurs at moderate levels across the landscape, with regional variation in introgression patterns. Dingoes in remote regions (e.g., Big Desert and Western Australia) exhibited lower levels of dog introgression, while populations in the East

and South showed higher rates of introgression, correlating with proximity to human settlements, anthropogenically modified landscapes, and the long-term, widespread implementation of lethal control programs in these areas. Key environmental variables, including the maximum temperature of the warmest month, the mean temperature of the driest quarter, and the Human Footprint Index, emerged as significant predictors of hybridization incidence. These findings suggest that human activity and environmental conditions play a crucial role in shaping hybridization dynamics between dingoes and domestic dogs, highlighting the complex interplay between hybridization, environmental pressures, and local adaptation in dingoes. Our findings provide critical insights for conservation strategies aimed at mitigating the impacts of hybridization on this apex predator in human-modified landscapes.



Domestication Reduces Plant Immune Receptor Gene Repertoires Across Lineages

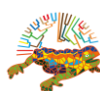
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MEETING ROOM 116, August 19, 2025, 11:00 AM - 1:00 PM

Plant domestication is sometimes associated with a reduction in the diversity of immune receptor genes (IRGs), critical for pathogen recognition and defence. Yet, the extent and evolutionary forces driving this pattern remain unclear. Using a comparative genomics framework, we analysed the IRG repertoires of 15 domesticated crop species and their wild relatives, representing nine plant families. We examined both cell-surface pattern recognition receptors (PRRs) and intracellular nucleotide-binding leucine-rich repeat receptors (NLRs). Our results show that five crops—grapes, mandarins, rice, barley and yellow sarson—exhibited

significantly reduced IRG repertoires compared to their wild counterparts; however, the overall rate of IRG loss reflected the background rate of gene loss. Despite this, there is a positive association between domestication duration and IRG loss. Together these results suggest that domestication imposes a subtle, cumulative pressure, consistent with relaxed selection rather than a strong cost-of-resistance effect. This study provides insights into how domestication impacts plant immunity, with implications for future crop breeding strategies to enhance disease resistance.



Dressed for survival: Positive survival selection on plumage colouration revealed by modelling labile trait expression

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Colour traits are commonly viewed as signals in sexual and social interactions, expected to reliably reflect key aspects of individual quality and predict their fitness. Measuring selection on colouration across different fitness components is thus key to appropriately assessing their reliability and understanding their evolutionary and ecological functioning. Yet, while most research has focused on reproductive success, associations between colouration and survival remain poorly studied, notably due to methodological challenges arising from the high lability of colour traits (i.e., within-individual variation across life) and non-random observation failure in the wild. Consequently, we still cannot fully understand how colouration predicts individual fitness, and how natural selection shapes the evolution of colour signals.

Accordingly, we developed advanced Bayesian analyses combining capture-recapture models (CRMs) with linear mixed models (LMMs). LMMs modelled labile variation in individual trait expression and allowed imputing uncertainty from missing observations into CRMs, enabling estimation of survival selection while controlling for phenotype-biased detection. We fitted these innovative models to 19 years of resighting data and ~10,000 plumage colouration measurements from ~3,000 individuals in two populations of blue tits (*Cyanistes caeruleus*), inhabiting contrasted habitats: a deciduous forest and an evergreen forest. This system provided unique opportunities to address how selection varies given the ecological context. We focused on two traits involved in mate choice and social

competition but differing in eco-physiological bases: ultraviolet chroma of the blue crown and carotenoid chroma of the yellow breast.

Preliminary simulations and model comparisons showed that our novel method outperformed previous approaches that neglected trait lability and associated observation biases. Moreover, we found that UV chroma was strongly positively associated with survival in both habitats, supporting its role as a reliable quality signal. In contrast, yellow chroma predicted higher survival only in the evergreen habitat, suggesting that carotenoid availability or habitat-specific selective pressures may drive differential survival benefits and influence the signalling function of carotenoid-based traits. We found no evidence for quadratic selection on either trait. Additionally, both traits were negatively associated with capture probability, indicating that more colourful individuals may be under-sampled due to lower capturability or site fidelity, highlighting the need to account for non-random missing data.

Overall, our study emphasises the need for robust analytical frameworks to detect selection on labile traits and reveal strong, context-dependant survival selection that we would not have detected otherwise. We highlight the important role of survival selection on ornamental traits, offering new insights into the ecological function and evolution of sexual and social signals.



Dual roles of DNA methylation in gene regulation and chromatin structure of a hemimetabolous insect

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Gene body methylation is one of the most taxonomically widespread epigenetic marks, yet its function and evolutionary history are poorly understood. While there is a general positive association between gene body methylation and gene expression, the mechanisms behind this relationship are unclear. Here, we investigate the function of gene body methylation in invertebrates, using the hemimetabolous insect *Timema cristinae*. We compare patterns of gene body methylation (WGBS), to gene expression (RNA-seq), open chromatin peaks (ATAC-seq), and chromatin compartments (HiC), across the genome. We find that 95% of

genes with gene body methylation occur in open chromatin compartments (euchromatin). Furthermore, highly expressed genes are greatly impoverished in methylation at their transcription start site (TSS), associated with a peak of open chromatin. Our findings suggest that observations of a positive correlation between gene body methylation and gene expression are due to the implication of gene body methylation in chromatin compartment formation. Yet, methylation around the TSS does regulate gene expression, playing an inhibitory role as in vertebrates and plants.



Dysploidy and demography: Chromosome number variation shapes population genomics in *Nicotiana* section *Suaveolentes*

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¹University of Vienna, ²Royal Botanic Gardens, Kew | Kew

MEETING ROOM 116, August 21, 2025, 2:00 PM - 5:00 PM

Chromosome number variation is a widespread phenomenon in angiosperms, often linked to processes such as polyploidy, dysploidy, and chromosomal rearrangements. Dysploidy, the gain or loss of individual chromosomes, can influence genome architecture, meiotic stability, and gene regulation, and is thought to play a significant role in plant diversification and speciation. To investigate the genomic consequences of chromosome number variation through dysploidy, we focused on *Nicotiana* section *Suaveolentes*, a clade of allotetraploid origin that originated approximately 6 Mya. Following polyploidization, this group underwent a progressive reduction in chromosome number (from $n = 24$ to $n = 15$). This group provides a powerful model to assess how chromosomal instability may influence patterns of population structure in contrast to closely related lineages with stable chromosome numbers. Using 305 RADseq samples across populations of *Suaveolens* and its sister clade *Occidentalis*, we tested whether chromosomal variability correlates with population connectivity, genetic diversity, and demographic history during the past two million years of aridification and ecological shifts in Australia. NGSadmix clustering analyses ($K = 2-10$) identified $K = 7$ as the best-supported model for both clades, confirming the presence of seven distinct populations in each group. Principal component analysis (PCA) further revealed strong genetic structure in *Occidentalis*, with populations forming discrete clusters across PC1–PC3. In contrast, *Suaveolens* populations overlapped in PC1–PC2 and separated only partially higher PCs, consistent with more diffuse structure and higher genetic connectivity. Pairwise F_{ST} estimates revealed markedly higher genetic differentiation in *Occidentalis*, with values reaching up to 0.505, indicating significant divergence and limited gene flow. In contrast, *Suaveolens* populations were more genetically cohesive, with lower F_{ST} values often below 0.3.

TreeMix analysis supported this dynamic: for *Suaveolens*, a model with three migration edges ($m = 3$) best explained the data, while for *Occidentalis*, a more conservative model with two edges ($m = 2$) was optimal. Inbreeding coefficient (F) estimates revealed a marked contrast between clades. *Occidentalis* populations exhibited high inbreeding levels (e.g., Burk mean $F = 0.61$), while *Suaveolens* showed substantially lower or even negative values (e.g., Maral $F = -0.067$). These findings support the hypothesis that chromosomal stability in *Occidentalis* may be associated with restricted gene flow and elevated inbreeding, whereas chromosomal variability in *Suaveolens* corresponds with broader connectivity and reduced inbreeding. Demographic modeling with fastsimcoal2 supported current migration as the best-fitting model for most population pairs in both clades. In *Occidentalis*, despite strong genetic structure, traces of recent migration were also supported in some population comparisons, suggesting occasional connectivity in an otherwise isolated framework. Stairway Plot analyses revealed distinct demographic trajectories: *Occidentalis* experienced a pronounced bottleneck around 100,000 years ago, coinciding with its diversification. *Suaveolens*, in turn, showed a major bottleneck around 80,000 years ago, with evidence of additional, more recent bottlenecks in specific populations. These contrasting demographic events align with differences in chromosomal architecture and their influence on long-term genetic stability and flexibility. Together, our findings show that chromosomal variation plays a fundamental role in shaping population structure, gene flow, and diversity. While *Occidentalis* exhibits compartmentalized populations with high differentiation and inbreeding, *Suaveolens* maintains broad allelic diversity, lower differentiation, and dynamic demographic patterns facilitated by its chromosomal variability.



Ecological drift drives variation in the microbiome and its effects on the red flour beetle

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Beetles are the most diverse insects on the planet, and it has been suggested that this diversity was facilitated by their associations with beneficial bacterial symbionts (Salem et al, 2022). While many cases of obligate symbiosis are well understood, the maintenance and assembly of facultative bacterial associates are underexplored. Here, we study an interesting model system, the red flour beetle *Tribolium castaneum*, an important pest for which cereal flour (typically wheat flour) serves as both habitat and food (i.e. all beetle life stages consume and live in flour). Earlier work in the lab showed that the beetle microbiome affects host fitness (Agarwal et al, 2020), but these effects were not repeatable in subsequent work. Surprisingly, the beetle microbiome composition and load fluctuated dramatically across time, individuals, generations, and replicate populations; though some bacterial genera such as *Izhakiella*, *Enterococcus*, *Acinetobacter*,

and *Pseudomonas* recur as core microbiome members. Testing several hypotheses underlying the observed microbiome variability, we find that life stage, age, source, and amount of fecal inoculum in the flour generate ecological drift during the assembly of the beetle's microbiome. Crucially, our laboratory maintenance protocol exacerbates this drift, explaining the observed variation in the beetle microbiome. Finally, our manipulative experiments show the differential colonization success of these bacteria, and the beneficial effects of *Izhakiella* and *Enterococcus*, suggesting that these might be the potential symbionts whose abundance in the microbiome determines the fitness consequences for hosts. Altogether, our work thus offers new insights into the drivers of ecological drift during microbiome community assembly and its implications for host populations.



Environmental dependence of local adaptation and expansion load across the range of an annual plant

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Theories on ecological and evolutionary drivers of range limits yield contrasting predictions about the relative performance of populations across species ranges. On the one hand, populations growing in different environments across species ranges may be locally adapted; local populations are thus expected to outperform foreign populations, and local adaptation may also prime edge populations for environments beyond range margins. In contrast, a history of range expansion may result in reduced performances of edge populations, because of the accumulation of deleterious mutations due to genetic drift and inbreeding depression – a process known as expansion load. Under this scenario, edge populations are expected to perform less well than non-edge populations that have higher genetic diversity, though the amount of expansion load may vary with post-expansion gene flow and its expression with environmental conditions. We tested these contrasting hypotheses using populations of the annual plant, *Mercurialis annua*, that underwent a

range expansion from the eastern Mediterranean Basin throughout central and western Europe. We transplanted populations from the “refugium”, “core” and “edge” populations into ten common gardens across and beyond the species’ current range. At the end of a single generation of growth, we measured various life-history and morphological traits and estimated population growth rates. Our results point to local adaptation in two gardens, but also fitness differences consistent with an effect of expansion load in five other gardens, where refugium populations with the highest genetic diversity outperformed other populations. Demographic rates and life-history traits at the early life stages, including germination behaviour and seedling survival, explained most of the observed variation in population performance. In summary, our study highlights the environmental dependence of local adaptation and expansion load and the important contributions of early life stages to population performance.



Evolution of developmental bias explains divergent patterns of phenotypic evolution in two nematode clades

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Rates of phenotypic evolution vary across traits, and these evolutionary patterns themselves

evolve. Understanding how development biases such patterns remains a challenge because it requires large-scale measurement of phenotypic variation resulting from random mutations across multiple species and clades.

Here, we tested whether divergent phenotypic evolutionary rates are explicable by the evolution of developmental bias using a quantitative genetics approach. We quantified in two clades of nematodes the relationships among phenotypic variation generated by de novo mutation, standing genetic variation, and evolutionary rates among species. For this, we used the homologous cellular framework of the six vulval precursor cells, named P3.p to P8.p, in *Caenorhabditis* and *Oscheius* species that diverged around 160 million years ago. Our phenotypic analysis of vulva cell fates on over 85,000 specimens quantified the evolution and mutational sensitivity of six cell fates across two clades. First, we find

that the cell whose fate evolves fastest is distinct for each clade: P3.p in *Caenorhabditis*, and P4.p in *Oscheius*. Second, we show that this evolutionary pattern matches the pattern of variation within species of each clade. Third, we find that, within each clade, the differential effect on each cell to random mutation, quantified as their mutational phenotypic variance, is sufficient to explain the observed phenotypic evolution. Finally, we propose that the difference between clades is explained by a simple spatial shift across cells of the sensitive region in a Wnt dose-response curve.

Altogether, we show that the evolution of developmental biases predicts more than 160 million years of divergent phenotypic evolutionary rates. These findings underscore the importance of integrating the evolving structure of the genotype-phenotype map and measures of developmental sensitivity into the understanding of phenotypic change at both micro- and macroevolutionary scales.



Expanding the genomic landscape of Capuchino seedeater speciation using a pangenome approach

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Rapid phenotypic diversification in the Capuchino Seedeaters (*Sporophila*) has been linked to the reshuffling of small regulatory alleles, particularly near melanogenesis genes, contributing to species differentiation. However, previous studies have largely relied on SNP-based approaches and a single reference genome, potentially missing structural variants (SVs) that may also play a role in divergence. To address this, we constructed a 32-haplotype pangenome using long-read sequencing and integrated it with short-read whole-genome sequencing from ten species to characterize genome-wide variation. This approach confirmed that differentiation is primarily driven by non-coding SNPs within narrow outlier regions enriched on the Z chromosome, reinforcing the role of regulatory variation in plumage evolution.

While we detected numerous SVs across the genome, we found no strong evidence of species-specific SVs in differentiation peaks, except for a single 55 bp deletion near *HERC2/OCA2*. Additionally, our results reveal that ~30 million variants present in the pangenome were undetected when using short-read data, likely due to mapping limitations in repetitive and complex regions. This suggests that key structural variants contributing to species divergence may remain overlooked. By leveraging a pangenome, we improve upon previous genomic resources and recover additional variation missed in single-reference based studies, underscoring the need for long-read sequencing of more individuals to fully resolve the genetic basis of rapid speciation in this system.



Female lineages and changing mortuary practices in Neolithic Çatalhöyük: Insights from genomic and archaeological data

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Ancient DNA can reveal patterns of genetic kinship among prehistoric burials, and when combined with archaeological data, it can shed light on gender-based mobility and social organization in past societies. The Neolithic site of Çatalhöyük, located in the Konya Plain of Anatolia, is well known for its large size, prolonged occupation, and abundant traces of complex symbolism. We co-analyzed whole-genome and archaeological data from burials spanning the early (7100–6700 BC), middle (6700–6500 BC), and late (6500–6300 BC) periods of Çatalhöyük East Mound to investigate house-based social organization and gendered social relations. In the early period, individuals were often buried with close relatives, but this pattern declined during the

middle and late periods. Instead, children from multiple families were buried in the same building and shared similar diets as indicated by strontium and oxygen isotope analysis. Furthermore, burials within the same buildings were more often related through maternal rather than paternal lineages and grave goods were significantly more common in the burials of subadult females compared to male subadults. Our findings illustrate how kinship relationships evolved through time, while certain mortuary practices emphasizing female lineages may have continued to shape the house-based social structure of Neolithic Çatalhöyük. This pattern of social dynamics persisted even amidst changing kinship practices.



From Molecular Promiscuity to Neural Specialization: Evolutionary paths to Nervous System Complexity

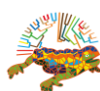
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The emergence of neurotransmission represents a pivotal innovation in animal evolution, yet our understanding of when and how these molecular signaling systems first evolved remains incomplete. Classical views suggest that complex neurotransmitter systems evolved specifically within Bilateria, and earlier-diverging animal lineages possess only basic signaling capabilities. There is growing evidence, however, that the monoaminergic and cholinergic systems were already in place in a pre-bilaterian and even pre-neuronal context. Using an integrative approach combining comparative genomics, phylogenetics, single-cell transcriptomics, structural modeling, and targeted metabolomics, we have found compelling evidence challenging this bilaterian-centric paradigm. Our research reveals the presence of diverse biogenic amines—including dopamine, histamine, tyramine, tryptamine, and acetylcholine—in non-bilaterian metazoans such as sponges and cnidarians. Phylogenetic analyses of key enzyme families involved in neurotransmitter biosynthesis and transport demonstrate that the molecular machinery for biogenic amine signaling predates the cnidarian-bilaterian split, originating from promiscuous enzymes and transporters initially serving non-neural functions already in the microbial Opisthokont ancestor. This work identifies a fundamental evolutionary trend from generalist to specialist machinery driving neurotransmitter system evolution. Specifically, I will show how acetylcholine first emerged as a metabolic byproduct of

carnitine-acetyl shuttling in energy-intensive cells, with subsequent co-option for signaling. Notably, muscarinic acetylcholine receptors and nicotinic channels expressed in both neurons and muscle cells were already present in the cnidarian-bilaterian common ancestor, demonstrating that cholinergic interneuronal and neuromuscular signaling evolved much earlier than previously recognized. Single-cell transcriptomics analysis further reveals that while bilaterian neurotransmission involves specialized, cell-type-specific synthesis and transport machinery, non-bilaterians achieve chemical signaling through more promiscuous enzymes with broader substrate specificities. The molecular specialization observed in bilaterians—including the duplication of carnitine acetyltransferase to produce choline acetyltransferase, and the diversification of vesicular transporters—represents a later evolutionary refinement rather than the origin of these signaling systems. Our findings fundamentally reshape our understanding of nervous system evolution, suggesting that the molecular foundations of complex neurotransmission were established in the earliest metazoans, with subsequent lineage-specific refinements enhancing specialization and efficiency. This "opportunistic evolution" model, where metabolic byproducts were co-opted for signaling and gradually specialized, provides a new framework for understanding the stepwise emergence of complex neural systems across animal phylogeny.



Functional components of neurotransmitter synthesis and transport were present in the ancestor of Animals and Fungi

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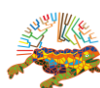
The appearance of neuronal cell types paved the way for complex responses to environmental changes and behavioral adaptations in animals. Neuronal cells emerge through the integration and co-option of a diverse protein machinery. Previous studies have shown that neuronal architecture is highly modular and individual components can be found in non-neuronal cell types or animals lacking neurons, where they perform their respective functions (e.g. calcium regulated vesicle secretion, electrical conduction and synaptic scaffolding–cellular contacts). Moreover, components homologous to neuronal secretory vesicle machinery have been shown to reside in intracellular, synaptic-like, vesicles in choanoflagellates, close unicellular relatives of animals. Thus some functional components of neurons emerged before the divergence of Metazoa. However, no previous studies have systematically addressed the existence and function of “neuronal” protein homologs outside animals and their close unicellular relatives. Our phylogenetic analysis indicates that homologs of synaptic vesicle transporters and neurotransmitter synthesizing enzymes are widespread in fungi, which, together with animals and their unicellular relatives, form the clade Opisthokonta. By functionally annotating these proteins and comparing them with their animal homologs, we investigate the timing of function acquisitions that implicated them in neurotransmission within Metazoa.

Firstly, using *Aspergillus nidulans* as a model system, we functionally characterised the orthologs of SLC18, synaptic vesicle, neurotransmitter transporters in Fungi. Employing GFP-tagging and colocalization assays, we show that they localise primarily to the endolysosomal system and, secondarily, to the plasma membrane. We also demonstrate their ability to transport substrates towards the vacuole (lysosome)/extracellular space,

conferring chemical resistance to strains overexpressing them. Taking into account functional data for bilaterian SLC18 transporters and the continuum between endosomal and synaptic vesicle membranes, we propose that the SLC18 of the Opisthokonta common ancestor was part of an endolysosomal system that expanded to include synaptic vesicles, within the metazoan lineage. The already present directionality of transport was employed for neurotransmitter loading.

Secondly, we identified and characterised the fungal ortholog of bilaterian aromatic amino acid decarboxylases (AADC), which are implicated in monoamine biosynthesis and a homolog of bilaterian choline acetyltransferases (ChAT), that mediate acetylcholine synthesis. Like their bilaterian counterparts, both GFP-tagged enzyme versions are cytoplasmically localized. We subjected strains overexpressing each enzyme to targeted metabolomics and compared the results to isogenic controls. We show that the fungal AADC is highly promiscuous and can catalyse histamine, tyramine and tryptamine synthesis which are major insect neurotransmitters and vertebrate neuromodulators. Additionally, the fungal ChAT homolog exhibits acetylcholine synthesizing capacity, which is the mediator of the vertebrate neuromuscular junction. Based on parsimony, monoamine and acetylcholine synthesis functions, homologous to neuronal ones, were present in the Opisthokonta common ancestor. Taken together, our results demonstrate that transport and metabolic functions, central to neuronal monoaminergic and cholinergic systems, were present in the unicellular ancestor of animals and fungi.

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Functional fate of duplicated genes

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Gene duplication is a major evolutionary source of functional innovation. Following duplication events, gene copies (paralogues) can be retained with functional modifications (such as silencing, sub-functionalisation, or neo-functionalisation) or loss. When paralogues are retained, this results in complex orthology relationships, including one-to-many or many-to-many. In such cases, identifying which paralogue is more likely to retain ancestral function remains a challenge. The “Least Diverged Orthologue (LDO) Conjecture” proposes that the paralogue with the slower evolutionary rate is more likely to maintain ancestral function.

This study explores this conjecture, using a novel method to identify asymmetric

evolution of paralogues and apply it to all gene families across the Tree of Life in the PANTHER database. Structural data for over 1 million proteins and expression data for 16 animals and 20 plants were then used to investigate functional divergence following duplication. This analysis, the most comprehensive to date, revealed that whilst the majority of paralogues display similar rates of sequence evolution, significant differences in branch lengths following gene duplication can be correlated with functional divergence. Overall, the results support the least diverged orthologue conjecture, suggesting that the least diverged orthologue (LDO) tends to retain the ancestral function, whilst the most diverged orthologue (MDO) may acquire a new, potentially specialised, role.



Gene expression profiling across the three-host life cycle of *Schistocephalus solidus*: how decoupled are developmental stages?

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Complex life cycles (CLCs) are a widespread and yet evolutionarily challenging strategy among parasites. Parasites with CLCs infect multiple hosts sequentially, adapting to distinct ecological niches but using information encoded within a single genome. While the evolutionary advantages for such a lifestyle are clear, a central question remains on how such a strategy evolved. Are the same genes expressed across different hosts when parasite stages perform similar functions—such as growth in both intermediate and definitive hosts—or does each stage utilise distinct gene sets for its specific environment and task?

The adaptive decoupling hypothesis proposes that different stages in a complex life cycle should be independent, under many points of view, allowing selection pressures in one host to act without repercussions in other phases of their lives. This absence of trade-off is crucial in evolving traits that are free to specialise in different niches.

We tested this hypothesis using a tapeworm, *Schistocephalus solidus*, by sampling transcriptomes throughout its complex life cycle and producing the biggest gene expression dataset for this species (57 transcriptomes from 10 *S. solidus* stages). Gene expression analysis was used to identify genes that are differentially expressed between hosts (e.g. 1st host vs 2nd host), between parasite functional stages (e.g. 'transmission' vs 'growing'), and between conditions (e.g. 'growing' in 1st host vs 'growing' in 2nd host). Gene set enrichment analysis further explored the main biological tasks in each life stage and the functionally similar stages were compared to verify if shared tasks were encoded by the same genes.

The strongest expression correlations were observed between stages that are temporally adjacent, even stages from different hosts and performing different tasks (e.g. waiting to be transmitted in one host vs invading the next) suggesting that gene expression patterns change slowly through the life cycle. For the non-consecutive stages, we found a lack of positive correlation in gene expression, even when those stages occur in the same host or perform similar biological functions, and this is supportive of decoupling: when some genes were upregulated in one stage, they were downregulated or not differentially expressed in others.

The gene set enrichment analysis revealed that even functionally similar stages (e.g. transmission in the copepod vs transmission in the fish) were characterized by different biological processes. In the case some processes matched in functionally similar stages, they were encoded by different genes, which uncovers another layer of decoupling: same biological processes but different gene sets used.

These findings provide compelling evidence for the adaptive decoupling hypothesis in parasitic worms. Each life stage of *S. solidus* exhibits distinct gene expression patterns, supporting the idea that stage-specific regulation enables these parasites to optimize performance across multiple environments. This mechanistic decoupling likely contributes to the evolutionary success of parasites with complex, multi-host life cycles.



Gene expression variability and evolutionary outcomes after whole-genome duplication in fishes

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Whole-genome duplication events (WGD) are often implicated in the evolution of functional diversity of the genome, yet paralogs retained after WGD (ohnologs) are known to be typically under strong selective or dosage constraints. This paradox between evolutionary novelty and constraint driving duplicate gene retention remains unresolved. Gene expression patterns have been widely used to investigate both biases in ohnolog retention and functional divergence after duplication, with studies focusing on average expression levels across organs. Recently, expression variability (i.e., the propensity of expression variation among individuals) has emerged as an important characteristic of genes, with both functional and evolutionary consequences. Here, we tested whether expression variability can predict evolutionary outcomes of ohnologs following WGD, using spotted gar (*Lepisosteus oculatus*) and Northern pike (*Esox lucius*) as outgroups to the teleost (230 – 320 Mya) and salmonid (88 – 103 Mya) WGDs, respectively. By comparing variability in outgroup species to evolutionary

outcomes in WGD lineages, we reveal that expression variability predicts both biased retention and functional divergence of ohnologs. First, we observed that single-copy orthologs of gene pairs retained after genome duplication have more variable expression relative to orthologs of singletons, following both the teleost and salmonid WGD. This effect is driven primarily by organ-biased genes rather than broadly expressed genes, which are preserved under strong selective constraints. Second, we observed that expression variability is associated with functional divergence of lowly constrained ohnolog pairs, suggesting that it can facilitate the exploration of phenotypic space following a duplication. Together, these results highlight intra-specific expression variability as a biologically meaningful lens to study the evolvability of duplicate genes over macro-evolutionary scales. Our findings thus show a dual role for selective constraint and expression evolvability in shaping the post-duplication trajectories of ohnologs.



Genetic & evolutionary mechanisms underlying phenotypic variation in a sexually selected trait

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A phenotype results from the interplay between an individual's genotype, its environment, and their interaction. Phenotypic variation refers to the range of phenotypic values that a trait can have within a population, and exaggerated sexually selected traits are among the most phenotypically variable traits in nature. However, the genetic and molecular mechanisms underlying the origin and maintenance of such extreme variation remain poorly understood. In the water strider *Microvelia longipes*, males have evolved exaggerated third pair of legs used in intrasexual competition, providing a promising system to investigate the genomic architecture of trait exaggeration under sexual selection. To identify alleles associated with phenotypic variation and to characterize the genomic response to sexual selection, I used an Evolve & Resequence (E&R) approach. Experimental populations were subjected to directional selection for long-legged males over 15 generations. At multiple time points, individuals from both selection and control lines were phenotyped for body size and leg length, and pools of 50

individuals per population were sequenced. It allows us to monitor both phenotypic evolution and allele frequency changes across generations. Phenotypic data show a clear response to selection: males from the selection lines exhibit increased leg size compared to the controls. Interestingly, phenotypic variation remains high in the selection lines, while it decreases in the controls, where no selective pressure was applied. These patterns suggest that sexual selection seems to maintain rather than erode phenotypic variation. The genomic analyses are ongoing; preliminary results will be presented to explore the genetic architecture and signatures of selection linked to trait exaggeration. This study aims to bridge the gap between phenotypic and genomic responses to sexual selection by combining experimental evolution with genomics. Our results support the idea that sexual selection can maintain high levels of phenotypic variation. The genomic analyses will shed further light on the evolutionary mechanisms driving and maintaining exaggerated sexual traits.



Genetic architecture of male reproductive success in a lekking bird: insights from predicted deleterious mutations

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Deleterious mutations are ubiquitous in natural populations and, when expressed, reduce fitness. However, the specific nature of these mutations and the ways in which they impact fitness remain poorly understood. We leveraged recent advances in genomics to predict deleterious mutations in the black grouse, an iconic lekking species. Analysis of 190 whole genomes alongside comprehensive life history data including repeated measures of behavioural, ornamental and fitness traits revealed that deleterious mutations identified through evolutionary conservation and functional prediction are associated with reduced male lifetime mating success. We assessed

whether both homozygous and heterozygous deleterious mutations affect fitness, which illuminated the dominance of mutations contributing towards an individual's realized mutation load. We further revealed how mutations in some genomic regions have disproportionately negative fitness effects. Finally, we assessed whether deleterious mutations impact male mating success through their effects on ornamental traits and sexually selected behaviours. Our findings offer novel insights into the genetic architecture of male fitness and illuminate the complex interplay between genetic variation and phenotypic expression.



Genetic architectures preventing species fusion between the Japanese sticklebacks

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Species boundaries are often permeable. Before complete reproductive isolation is established, hybridization and introgression frequently occur. Although hybridization can sometimes lead to hybrid swarms and speciation reversal, in other cases, species fusions can be prevented. What genetic architectures enable such permeable but robust species barriers? Recently, we have found a clear case that the species fusion was prevented by rapid purging of one species' genome within 10 generations after hybridization between Japanese sticklebacks, freshwater-resident *Gasterosteus aculeatus* and anadromous *G. nipponicus*. The hybridization was induced by the devastating 2011 Tohoku earthquake and tsunamis. In order to understand what drove the purging, we investigated whether *G. nipponicus* alleles at major loci involved in reproductive isolation were purged. To this end, we conducted QTL mapping of ecological selection and habitat choice. In addition to the previously identified loci responsible for sexual isolation and hybrid sterility, most of the major loci responsible for survival and habitat choice in the tsunami-created hybrid habitat, also clustered on the X chromosome. We found that the *G. nipponicus* alleles at these major loci were more rapidly purged than other

genomic regions, indicating that these major reproductive isolation drove this process. Surprisingly, however, most of the other *G. nipponicus* alleles at autosomal loci were also purged within the 10th generation with an exception for a few genomic loci. To understand what can drive the genome-wide purging, we conducted individual-based simulations. We found that a few major loci alone cannot induce the genome-wide purging. This is because once the introgressed alleles at major loci are rapidly purged in the first few generations, no more negative selection acts on the individuals carrying introgressed alleles. Instead, purging across the genome could be reproduced by a model with both a few major loci and many minor negative epistatic incompatibilities (i.e., Dobzhansky-Muller incompatibilities; DMI). In this model, we also found that adaptive introgression is possible while the majority of the introgressed alleles were purged. These findings suggest that a few major loci for reproductive isolation and many minor DMI loci enable permeable but robust species barriers. We are now attempting to validate these findings by investigating multiple natural populations and experimental evolution in outdoor mesocosms.



Genetic Patterns of Social Structuring in the Cuckoo Catfish

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The Cuckoo Catfish, *Synodontis multipunctatus* is a group living brood parasite in Lake Tanganyika, exploiting parental investment of maternally mouthbrooding cichlids. Multiple parentage clutches of parasitic offspring indicate that competition over reproduction is common. Considering inclusive fitness theory, group members of the same sex may gain indirect fitness benefits when choosing to group with their relatives if competition for reproductive opportunities cannot be avoided. Information on within-group relatedness in interspecific brood parasites have received little attention but can help to understand potential indirect fitness benefits of their group-living. While males primarily compete for fertilizations, females compete for access to host spawning and adoption by host female.

We collected genetic samples of Cuckoo Catfish groups from a wild population at Lake Tanganyika, including solitary individuals and groups of different sizes. Using relatedness estimates calculated from microsatellite allele frequencies, we found elevated relatedness among female group members, but not among males. Surprisingly, we also found slightly elevated relatedness between males and females of the same groups. Competition over reproduction is typically higher among males, as access to females typically limits male reproductive success. However, in this brood parasitic fish, females may face higher competition for spawning opportunities, as hosts rather than eggs become the limiting factor. This may ultimately lead to increased female relatedness within groups.



Genome-wide Population Structure of Lake Whitefish (*Coregonus clupeaformis*) in a Subarctic Great Lake.

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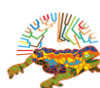
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Effective genetic monitoring of harvested species relies on our ability to delineate populations correctly. This can be challenging in large lacustrine or marine environments where gene flow tends to blur genetic structure. In Great Slave Lake, one of the world's largest freshwater lakes, Lake Whitefish has supported a commercial fishery since the 1940s. However, the genetic structure of populations was never assessed, and thus genetic monitoring was never implemented.

Using low-coverage whole-genome resequencing of 305 whitefish from ten sampling locations, we unraveled the presence of eight genetically differentiated populations of Lake Whitefish in Great Slave Lake. We discovered that populations were structured regionally in this Lake, with large bays and tributaries harbouring genetically different spawning populations. We also found fine-scale genetic structure between populations in the Western part of the lake, with relatively elevated levels of genetic differentiation between populations in this region despite small geographic distances (<100km). This suggests that mechanisms

such as local adaptation, spawning site fidelity or spatial ecology might influence Lake Whitefish population structure in this system. Furthermore, using genome-wide scans, we found that most genetic differentiation between populations was observed in genomic islands of divergence located in putative structural variants such as inversions. These results highlight the role of chromosomal rearrangements in the maintenance of locally adapted populations in environmentally heterogeneous lakes in the face of gene flow.

Overall, our study provides novel insights into the genetic structure of fish populations in vast, recently deglaciated lakes of North America. Our results highlight the power of whole-genome sequencing data for population delineation in systems with high gene flow, as genome-wide scans can facilitate the detection of genomic islands of divergence. Lastly, our precise assessment of genetic structure will provide an accurate population baseline for the future genetic monitoring of socio-economically important Lake Whitefish commercial fisheries in Great Slave Lake.



Genomic insights into the evolutionary potential of sharks in a changing ocean

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The ocean is changing rapidly, with rising sea temperatures and declining levels of dissolved oxygen posing major challenges for marine life. However, the capacity of many species to respond to these shifts remains poorly understood. This is particularly relevant for some shark species that are ectothermic- relying on external conditions for thermoregulation- and active predators with high oxygen demands. The blue shark (*Prionace glauca*) is especially exposed to these combined pressures. In addition to these physiological traits, it is the most heavily fished shark species globally, making it an ideal model for investigating how a pelagic predator under intense environmental and anthropogenic stress can adapt and persist. Yet, how these pressures affect the species remains unclear, largely due to limited knowledge of its genetic diversity. Early microsatellite studies based on nursery areas suggested a genetically homogeneous, panmictic population across the Atlantic. More recent SNP-based analyses, however, identified signals of possible differentiation between the Atlantic and the Mediterranean, and within the Atlantic. While informative, these approaches offer limited resolution to detect fine-scale structure or demographic trends. Whole-genome sequencing is therefore crucial to clarify these patterns and assess the species' adaptive potential with greater resolution.

Our research aims to provide a comprehensive understanding of the genomic diversity and evolutionary history of the blue shark through three complementary components. First, we are generating a high-quality, chromosome-level reference genome from a North Atlantic individual to support comparative and population-level analyses. Second, to establish a macroevolutionary framework, we are investigating the evolution of three traits linked to environmental adaptation- diving behaviour, thermal niche, and metabolic rate. These traits have evolved repeatedly across shark phylogeny, providing a unique opportunity to detect genomic signatures of convergent evolution and identify candidate genes

associated with adaptation. These analyses are ongoing, and preliminary results for at least one trait are expected by the time of the conference. This comparative approach not only helps us reconstruct the evolutionary history of these traits, but also to assess whether the same genes may be under contemporary selection, potentially contributing to resilience in a changing ocean. Third, we focus on population-level analyses using whole-genome sequencing data for the first time in this species. Our initial objective is to investigate fine-scale genetic diversity and structure across the Atlantic, testing whether high mobility results in homogeneity or if population structuring is detectable. A second objective examines diving behaviour in hypoxic environments- specifically, the blue shark's reported use of Oxygen Minimum Zones (OMZs). By integrating genomic and individual-based movement data, we aim to test whether variation in behaviour correlates with genomic differences. This may help identify behavioural phenotypes with distinct adaptive capacities and inform predictions about the species' responses to future ocean deoxygenation. Preliminary population structure results suggest a latitudinal gradient in genetic differentiation between northern and southern Atlantic populations, providing the most robust genomic evidence to date of structure in this species. This pattern may reflect environmentally influenced barriers to gene flow or emerging local adaptation, and forms a basis for deeper investigation into the processes shaping spatial genomic variation.

This work combines macroevolutionary, population-level, and behavioural ecological perspectives to build a framework for understanding how pelagic predators migrate and adapt across spatial, physiological, and evolutionary dimensions. Ultimately, we aim to inform conservation strategies for this and other ecologically similar species in a rapidly changing ocean.



Genomics, Epigenomics, and Transcriptomic Insights into Hybridization and the Evolution of Reproductive Barriers in *Pitcairnia*, a Neotropical Bromeliad of adaptive radiation

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MEETING ROOM 116, August 21, 2025, 2:00 PM - 5:00 PM

Reproductive isolation between species is maintained by barriers acting before (prezygotic) or after (postzygotic) fertilization. This study investigates how structural variants, differential gene expression, and epigenetic regulation collectively drive the evolution of reproductive isolation and assortative mating in *Pitcairnia*—a model system for adaptive radiation in naturally fragmented Neotropical habitats. By integrating genomic, transcriptomic, and epigenomic approaches, we unravel the mechanistic basis of ecological speciation, demonstrating how these multi-layered molecular processes shape adaptive divergence and reproductive barriers. First, we assembled and annotated high-quality genomes for two species using PacBio HiFi and Omni-C sequencing. These assemblies were highly contiguous and syntenic, capturing over 98% of BUSCO genes, although six of the 25 chromosomes exhibited large inversions, ranging from 700kbp to 4.4Mbp. Next, we analyzed differential gene expression (DGE) and DNA methylation in sympatric and allopatric populations of *P. staminea* and *P. albiflos*, and their natural hybrids. Stranded RNA-seq data were generated using Illumina technology. SNPs were called and filtered using the ANGSD workflow, yielding over 500,000 high-quality polymorphic sites across 59 individuals. Bayesian clustering via NGSadmix identified two distinct genetic clusters corresponding to each parental species, with admixture detected only in sympatric populations. Of the 59 individuals analyzed, 21 were classified as pure *P. albiflos*, 22 as pure *P. staminea*, and 16 as hybrids with intermediate genomic assignment values. Gene expression analysis using FeatureCounts, edgeR, and DESeq2 revealed strong species-specific patterns. Principal Component Analysis (PCA) of normalized expression data showed distinct clustering of parental species with hybrids clustering in between. Few hybrids

displayed transgressive expression profiles, with gene expression beyond the range of either parent. We identified different gene expression profiles between pure species between the pure parental species, with enriched Gene Ontology (GO) terms associated with the pentose phosphate pathway, defense responses, abscisic acid signaling, and di-/triterpenoid biosynthesis—key pathways in responses to environmental stressors such as drought, waterlogging, and UV exposure. The distinct gene expression profiles of parental species, intermediate and transgressive expression in hybrids, and stress-related functional enrichment of DEGs support a regulatory basis for speciation, suggesting that regulatory divergence in stress-response pathways may contribute to ecological speciation and the maintenance of reproductive isolation between these species. Further, to assess epigenetic contributions to reproductive isolation, we conducted whole-genome enzymatic DNA methylation profiling in the same individuals. Although global methylation levels across CpG, CHG, and CHH contexts were similar, MethylKit identified differentially methylated regions (DMRs) between species, with allopatric populations showing fewer DMRs than sympatric ones. This result suggests that genomic interactions in hybrid zones may induce epigenomic instability or genome shock. Together, these findings show the critical role of regulatory divergence—through differential gene expression and epigenetic variation—in driving reproductive isolation and ecological speciation in *Pitcairnia* species. This study enhances our understanding of the molecular mechanisms underlying diversification in Neotropical plants and underscores the value of integrating transcriptomic and epigenomic data to advance ecological and evolutionary research.



Half Clone Full Complexity: Unexpected Genomic Diversity and Reproductive Strategies in Hybrid Water Frog Hemiclonal Population Systems Across Ukraine

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MEETING ROOM 115, August 21, 2025, 2:00 PM - 5:00 PM

Background: Water frogs from the Pelophylax esculentus complex, encompassing sexual species *P. ridibundus* and *P. lessonae* alongside their hybrids (*P. esculentus*), offers a compelling system for studying evolution of interspecific interactions, hybridization, polyploidy, and speciation. Hybrid frogs reproduce via hybridogenesis, a hemiclonal reproductive strategy where one parental genome is clonally transmitted and the other discarded, typically requiring a sexual host species for reproduction. Across Europe, hybrids mainly coexist with one or both parental species, forming L-E (with *P. lessonae*), R-E (with *P. ridibundus*) or L-E-R (with both species) hemiclonal population systems (HPSs). Additionally, some systems include triploid hybrids. Moreover, diploid and triploid hybrids can form all-hybrid (E-E) HPSs. Stability of water frogs populations relies on mechanisms including diverse fecundity, gametogenesis patterns in hybrids, and selective offspring mortality. While HPS in Central and Northern Europe have been well studied, Eastern Europe, and Ukraine in particular, remains underexplored. In this study, we analyzed composition, genomic diversity, and dynamics in Pelophylax HPSs across Ukraine.

Materials and methods: During the 2012-2025 we collected over 2000 water frogs from Dnipro and Siverskyi Donets River basins. Each individual was analyzed with a combination of two or more methods: morphometry, karyotyping, FISH, nuclear genetic markers (microsatellites, *uqcrrs1* gene).

Results: In Eastern Ukraine, within the basin of Siverskyi Donets River, we observed various R-E HPSs where *P. ridibundus* coexist with hybrids of different ploidy. The hybrid form prevailed in most populations. Surprisingly, we found that *P. esculentus* here produce both R- and L-gametes, even though we expected only L-gametes, as opposed to the R-gametes they produce in L-E HPS. This enables hybrid-hybrid crosses without parental species involvement. Such crosses, as well as hybrid × *P. ridibundus*, should yield a high portion of *P. ridibundus* among offspring. Comparing genotypes of

tadpoles and adult frogs in several distinct HPSs, we showed that these HPSs strongly depend on mortality of *P. ridibundus* and, sometimes, triploid offspring arising from hybrid-hybrid or hybrid-parental crosses. Microsatellite analysis revealed greater diversity in *P. ridibundus* genomes than in *P. lessonae* genomes within hybrids, likely reflecting the absence of *P. lessonae* as a main source of recombined genomes in the region. Accordingly, R-genomes exhibited significantly lower clonality than L-genomes. The presence of several clonal L-genome lineages may indicate their multiple origins in Eastern Ukraine.

In contrast, HPSs in North-Eastern, Central, and Western Ukraine (Dnipro River tributaries basins) often included both parental species along with diploid and triploid *P. esculentus*, forming the most complex water frog HPSs known. Chornobyl Zone HPSs were found to be much more variable than previously presumed, with a possible contribution of unique environmental to their diversity and evolution. Microsatellite data showed that L-genomes were generally more diverse, likely because *P. lessonae* was more often involved in hybrid reproduction. However, *P. lessonae* often occurs in low numbers, with historical data showing a significant decline in recent decades, a trend observed across Europe. Meanwhile, *P. ridibundus* dominates quantitatively, consistent with its Europe-wide northward expansion. Features of hybrid reproduction in these HPSs remain to be fully investigated, though the broad distribution of these populations suggests that their intrapopulation processes and origins may be highly distinct.

Conclusion: Revealing diverse reproductive strategies, unexpected genetic diversity, and population dynamics of such complex Pelophylax populations is crucial for understanding species interaction, selection processes, speciation evolution, and consequent successful conservation management.



How does microbial diversity affect ecological stability?

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MEETING ROOM 117, August 18, 2025, 2:00 PM - 5:00 PM

Background: Microbial communities, exemplified by the human gut microbiome, engage in complex symbiotic interactions that significantly influence ecological stability. Understanding the conditions under which species diversity stabilizes or destabilizes microbial ecosystems remains unresolved.

Materials and Methods: We constructed a novel theoretical model bridging classical ecological theory (gLV, consumer-resource, etc.) with microbial symbiotic interactions, incorporating nonlinear density-dependent growth functions that describe how per capita growth rates respond to microbial population densities. We explored this model through analytical and numerical simulations across a range of ecological scenarios representative of host-associated microbiome.

Results: Our theoretical analysis revealed that increased microbial diversity can

frequently destabilize microbial communities due to competitive interactions. Stability was only enhanced under narrow conditions characterized by particular forms of decelerating functions and low similarity between interspecific and intraspecific interactions. Our statistical analysis of microbial growth data further showed that destabilizing patterns are likely predominant in microbiome.

Conclusions: Our work offers a comprehensive reevaluation of the diversity-stability paradigm in microbial symbioses. By elucidating precise ecological conditions that govern stability, our study provides a mechanistic foundation for predicting and manipulating stability in microbial ecosystems, with implications for understanding the dynamics of host-associated microbiomes and their ecological resilience.



How models accounting for population structure can improve our understanding of the evolutionary history of common chimpanzees and bonobos

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MEETING ROOM 116, August 21, 2025, 2:00 PM - 5:00 PM

Most evolutionary studies of common chimpanzees (*Pan troglodytes*) have assumed that the four currently recognized subspecies can be modeled using simple tree models where each subspecies is panmictic. In addition, a recent piece of work has detected admixture between chimpanzees and their closest relatives, bonobos (de Manuel et al. 2016). However, several studies have identified the existence of significant population structure and patterns of isolation-by-distance in common chimpanzees, both within and between subspecies.

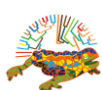
It has been recognized for some time now that ignoring population structure can lead to the inference of demographic events that never took place. Assuming panmixia when the species is in fact structured can lead to the detection of population size changes that could also be caused by structure and changes in connectivity between sub-populations (Heller et al. 2013, Mazet et al. 2016). In addition, some studies have shown that using models without population structure can lead to the detection of spurious introgression events (Eriksson and Manica 2012, 2014, Tournebize and Chikhi 2025).

Here, we propose two models that account for population structure to better understand the evolutionary history of chimpanzees and bonobos.

First, we focus on chimpanzees and use n-island models within each subspecies to infer a demographic history in terms of changes in connectivity. To that end, we use SNIF (Arredondo et al. 2021), a method developed to

infer a piecewise stationary n-island model using PSMC (Li and Durbin 2011) curves as summary statistics. We then propose a general model integrating the four subspecies as metapopulations within a phylogenetic tree. We find that this model correctly predicts estimates of within subspecies genetic diversity and differentiation, but overestimates genetic differentiation between subspecies which we recover by adding gene flow between subspecies.

Second, we use 1D stepping-stone models for each subspecies of chimpanzees and for bonobos to test if such models, which allow for gene flow between subspecies but do not allow for gene flow between bonobos and chimpanzees, could explain signals of introgression that have been found between these two species. We show that we are able to reproduce values of D-statistic, a common statistic used to detect introgression, close (and even higher) to the values found between chimpanzees and bonobos, suggesting that empirical signals of introgression could be explained by structure alone. Furthermore, our model manages to produce realistic values of nucleotide diversity and genetic differentiation. Altogether, our study shows that models integrating population structure can explain several patterns of genetic diversity observed in chimpanzees and bonobos, which calls for more caution when interpreting signals of population size changes and introgression events as such when studying structured species.



Indications of different sex determination systems in two closely related Eurasian minnow (*Phoxinus* spp.) species

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MEETING ROOM 131, August 19, 2025, 2:00 PM - 5:00 PM

Background. Its hypothesised that closely related species differ in sex determination systems, possibly to hinder hybridisation. We compared two closely related minnow species (*Phoxinus phoxinus* and *P. csikii*) that occur in proximity to each other—in the Rhine and Danube, respectively, which are connected via the Rhine–Main–Danube Canal, with evidence of hybridisation to test this assumption.

Materials and methods. We performed sex chromosome identification investigation using the SexFindR pipeline on approx. 10x Illumina whole genome re-sequencing data per individual. Thirteen males and females, each, of *P. phoxinus* and eight males and females, each, of *P. csikii* were included in the analysis. Mapping to a recently released annotated, chromosome-level reference genome for *P. phoxinus*, the samples were analysed regarding coverage differences, SNP-based differences and kmer-based (reference-free) GWAs.

Results. A coverage analysis revealed no differentiated (i.e. heteromorphic) sex chromosomes in both species. However, in *P. phoxinus* SNP-based analyses highlight two sex-associated regions, one on chromosome 3 (20kb) and one on chromosome 12 (10kb). The region on chromosome 3 is further corroborated by a reference genome-free kmer-based analysis and succeeds to differentiate males and females in a multidimensional scaling (MDS)

plot. Females are mostly homozygous in that region while males are heterozygous, matching a male heterogametic XY-sex determination system.

In *P. csikii*, we observed an impact of the underlying reference genome used for SNP-calling as the reference-free approach (kmers) separated males and females, when SNPs did not. The kmer-based approach supports two sex-associated regions on chromosome 3, as well, but in a different region than in *P. phoxinus*. The first region contains only female-specific kmers, not present in males; the second region separates males and females in a MDS plot. These results and genotype clustering of the second region indicates male-homozygosity and hence suggests a ZW-sex determination system in *P. csikii*.

For both species, gene ontology analysis shows the identified sex-associated regions contain genes that are directly linked to sex determination and differentiation or indirectly linked to gonadal development determination, such as *Figla*.

Conclusions. Two closely related species, *P. phoxinus* and *P. csikii*, likely have different sex-determining mechanisms, *P. phoxinus* an XY-system and *P. csikii* a ZW-system. The genomic investigation of hybrids should shed light on whether any mito-nuclear incompatibilities arise from this circumstance.



Individual sociability accelerates call combination learning in Western Australian magpies

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MEETING ROOM 118+119, August 19, 2025, 2:00 PM - 5:00 PM

Combinatoriality is the capacity to combine discrete vocal elements into higher order structures. Previously thought unique to human language, combinatoriality has now been documented in several non-human taxa. This has presented the opportunity for comparative research that could elucidate the evolutionary origins of syntax. The capacity for combinatoriality is believed to be related to social complexity and the need to transmit more complex information, as per the Social Complexity Hypothesis for Communicative Complexity (SCHCC). Thus far, research into combinatoriality is limited to studies that focus primarily on adult repertoires, limiting the ability for determining social influences on the emergence of such higher-order vocal sequences. We used longitudinal

observations of magpie fledglings to document the emergence of the call repertoire and investigate the effect of social complexity on both the speed at which call combinations are learnt, and repertoire size. We found significant social influences on the emergence of fledging call combinations, suggesting that call combinations are socially learnt, not innate. Specifically, fledglings who spent more time interacting with others produced combinations sooner in their development, and those that had a greater number of contacts produced more combination types across development. This study is the first to document the emergence of combinatoriality in an open-ended vocal learner, and to provide ontogenetic evidence that the SCHCC applies to combinatoriality.



Individual social bonds predict fitness in a cooperative bird

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MEETING ROOM 118+119, August 19, 2025, 2:00 PM - 5:00 PM

Understanding how sociality relates to individual fitness is key to uncovering the ecological and evolutionary dynamics of social behaviour. It is well known that forming groups can improve protection against predators or enhance foraging efficiency. More recently, data arising from long-term social-network studies suggest that the benefits of sociality arise not just from variation in group size, but also in the quality of individual associations. Yet surprisingly few long-term studies have quantified whether some measures of individual sociality are consistent through time and predict key fitness components. In this study, we investigate whether individual sociality levels remain stable over time and how they relate to survival and breeding probability in a population of sociable weavers (*Philetairus socius*), a highly cooperative bird. To address these questions, we quantified each individual's number of strong social bonds—defined as associations in the top 20 % of interaction

strength—and classified each bird into high, medium or low sociality levels. We estimated the stability of these sociality levels between seasons, and then examined their association with annual survival using multistate capture–recapture models, and with subsequent breeding probability using GLMMs. We found that (i) individuals were more likely to remain at the same sociality level over time, with any transitions tending to adjacent levels; (ii) birds at medium and high sociality levels exhibited up to 20 % higher annual survival probabilities than less social individuals; and (iii) high-sociality birds had up to 30 % greater breeding probability than low-sociality birds. Our results indicate that fine-scale, individual-level social interactions contribute substantially to fitness—beyond the benefits of group membership—highlighting additional axes of social selection in cooperative societies.



Intergenerational transfer of reproductive plasticity in fruitflies

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MEETING ROOM 115, August 21, 2025, 2:00 PM - 5:00 PM

Plastic responses of individuals to their socio-sexual environments can represent key determinants of fitness. For example, organisms exposed to an elevated threat of sexual competition may, in response, invest more in reproductive traits such as ejaculate volume or quality, thus increasing their relative fitness. An emerging body of research is revealing the considerable scale, scope and sophistication of such responses. The possibility that the plastic responses of parents could have anticipatory effects on the degree of behavioural plasticity expressed by their offspring, has received less attention. Here we addressed this omission using the fruitfly *Drosophila melanogaster*, which has become a useful model for testing the ultimate and mechanistic responses of individuals to their social environments. We tested the hypotheses that responses of mothers to their social environment would: (i) affect the extent of offspring behavioural plasticity, and (ii) have sex-specific effects on offspring. To do this, mothers were either socially isolated, or exposed to 3 other conspecific

females for 72h, before being separated to undergo single pair matings. Maternal mating latency, duration and the number of eggs produced in the subsequent 24h were then recorded. We then tested these same traits in daughters of isolated or grouped treatment mothers, following exposure to either social treatment, using a factorial design. For sons, we tested the effect of a mother's social environment on mating duration, measured under standardised conditions. The results showed that daughters of mothers maintained in social groups had greater plasticity in mating latency and laid more eggs than did daughters of socially-isolated mothers. There were no consistent effects of the maternal environment on the mating duration plasticity of sons. The results confirmed that the maternal social environment affects the extent of reproductive plasticity exhibited by offspring, and that these effects can be sex-specific. Transcriptomic responses of each sex to their social environments are starting to reveal the potential mechanisms involved.



Interplay between low-recombining regions and overdominance in a plant genome?

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MEETING ROOM 116, August 19, 2025, 2:00 PM - 5:00 PM

To what extent overdominance might contribute to the maintenance of genetic diversity within genomes is still an ongoing research question. Pseudo-overdominance – a particular case of overdominance – created by the complementation of deleterious alleles in heterozygotes has recently become a subject of particular interest. Simulations and theory suggest that pseudo-overdominance may occur in low recombining regions. We conducted a comprehensive investigation of low-recombining regions in cultivated populations of pearl millet, an outcrossing diploid African cereal. We studied seven large genomic regions ranging from 5 to 88 Mb. Six of these regions were pericentromeric. These regions exhibit an excess of heterozygotes, a distinctive hallmark of overdominance. They displayed a tendency towards a higher diversity and ratio of non-synonymous and deleterious

variants. We conducted a comprehensive study of the largest 88 Mb region identified on chromosome 3. Interestingly, haplotypes of this region have been introgressed from wild relatives. We resequenced multiple genomes and confirmed their strong divergence and the presence of inversions across one of them. One of the haplotypes seemed highly deleterious in the homozygous state. A total of 17% of the cultivated pearl millet genome exhibits a local population structure suggestive of overdominance or possibly pseudo-overdominance. Our empirical results contribute to the accumulation of knowledge that will enhance our understanding of the potential role of overdominance or pseudo-overdominance in maintaining genetic diversity, particularly in low recombining regions.



Investigating changing Wnt signal as a mechanism influencing differential evolutionary rates of vulval cell fates in nematodes.

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MEETING ROOM 132, August 18, 2025, 10:30 AM - 12:30 PM

Although mutation allows for random exploration of the genotypic space, realized phenotypes

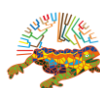
are often biased in particular directions of phenotypic space. This realized phenotypic variance resulting from mutation is termed as mutational variance. Observed phenotypic variance in wild populations results from natural selection acting on realized phenotypes. Many studies have documented the influence of both magnitude and direction of mutational variance on the rate and trajectory of evolutionary change. However, little is known about underlying mechanisms influencing the observed phenotypic variance. Developmental mechanisms are an excellent candidate for influencing phenotypic expression of morphologic traits, as the genotype-phenotype relationship is mediated in developmental space. We tested the simple hypothesis that different levels of a developmental signaling pathway mediate a differential phenotypic variance between two nematode genera.

Despite an invariant cell lineage touted as a primary advantage of the *Caenorhabditis elegans* model, variation exists for the fate of some vulval precursor cells (VPCs). The VPC competence group consists of 6 cells (P3.p – P8.p) arranged along the anterior-posterior axis. While all cells are competent to form the vulva under specific conditions, P3,4,8.p are not recruited in typical development. Instead, these cells take on one of two fates: fusing with the epidermis early in development or dividing once before later fusion. Wnt signals, expressed mostly from the posterior, induce cell division in VPCs. P3.p, the most anterior VPC, is most sensitive to Wnt dose variation and to random mutation in *C. elegans* and evolves

rapidly in the *Caenorhabditis* genus with high levels of variation within and between species. In *Oscheius*, another genus in the family Rhabditidae, P3.p fate is primarily undivided and it is the next most posterior cell, P4.p, that is rapidly evolving and most sensitive to random mutation (Picao Osorio et al. bioRxiv 2025). This posterior shift of the VPC in the sensitive zone is predicted to correspond to a change in Wnt between the genera.

We investigated whether a decrease in Wnt signal in *C. elegans* was sufficient to create a *O. tipulae*-like mutational variance pattern: fixed P3.p fate and variable P4.p fate. We applied random mutation to a *Cel-cwn-1* (Wnt) mutant and a wild-type control background, and assayed mutational variance of VPC fates in both contexts in over 20 mutation lines derived from each of the mutant and wild-type backgrounds. We found that the mutational variance pattern of the Wnt mutant does resemble that of *O. tipulae*, with variable P4.p fate. This observation provides evidence that differential Wnt signaling is sufficient to explain between the two clades different biases in cell fate variation and evolution.

We propose that changes to a shared developmental mechanism, specifically Wnt signaling, explain different evolutionary rates of vulval cell fates between *Caenorhabditis* and *Oscheius* nematodes. At the microevolutionary level, the cell in the Wnt-sensitive zone is that evolving fastest. At the macroevolutionary level between the two genera, this Wnt-sensitive zone shifts spatially between two cells along the antero-posterior axis, and this shift can also be explained by a change in Wnt signaling level. Thus, levels of a single signaling pathway explain both micro- and macro-evolutionary processes.



Junk or Dark DNA? Using a human-Arabidopsis hybrid cell line to realise the Random Genome Project at mega-base Scale

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MEETING ROOM 116, August 19, 2025, 2:00 PM - 5:00 PM

Background: The human genome is pervasively transcribed, yet most of the genome does not have a clearly characterised function. There is currently intense debate over whether this pervasive transcription is evidence for widespread functional ‘dark’ DNA or is simply a consequence of background transcriptional noise from junk DNA. To resolve this debate, Sean Eddy proposed the ‘Random Genome Project’ over a decade ago, a thought experiment where random DNA in a human cell is assayed to determine the baseline level of transcription. Here, we realise Eddy’s Random Genome Project at ~35 megabase scale by assaying transcription of a human cell line containing ~40% of the Arabidopsis genome.

Materials and methods: A previously established human cell line was reported to have a large part of the Arabidopsis genome inserted into a single site in human chromosome 15. Nanopore whole-genome DNA sequencing (WGS) was performed to confirm the presence of Arabidopsis DNA and to generate a custom reference that includes the Arabidopsis component of the hybrid cell line genome following assembly with Flye de novo. Illumina RNA-seq was then used to assay transcription of the hybrid cell line. These reads were aligned to the custom reference, as were reads from three publicly available Arabidopsis RNA-seq datasets. Patterns of read alignments to Arabidopsis exonic and intergenic regions were then analysed to determine which Arabidopsis regions are transcribed and at what levels.

Results: WGS confirmed the presence of over 35 MB of Arabidopsis DNA in the hybrid cell line. RNA-seq data analysis revealed widespread transcription of the Arabidopsis DNA. We compared Arabidopsis transcription between exonic and intergenic regions and found similar levels in both regions in the

hybrid cell, while, as expected, transcription was heavily biased to exonic regions in the Arabidopsis RNA-seq datasets. Moreover, Arabidopsis DNA transcription in the hybrid cell occurred over broader regions than observed in the native Arabidopsis RNA-seq datasets. Together, these results suggest that transcription in the hybrid cell is not being driven by Arabidopsis gene signatures and Arabidopsis DNA is a form of random DNA in human cells. Critically, we found that the distribution of transcripts is not very different in the Arabidopsis DNA compared to the native human genome in the hybrid cell line.

Conclusions: We found widespread transcription within a ~35 MB piece of Arabidopsis DNA present in a human cell, but this transcription does not align with canonical Arabidopsis features. This suggests that observed Arabidopsis transcription follows human rather than Arabidopsis transcriptional rules, and therefore is likely initiating from DNA signals that appear stochastically in Arabidopsis DNA. We conclude that Arabidopsis DNA is sufficiently evolutionarily distant to be uninterpretable to human transcriptional machinery, and therefore acts as random DNA in human cells. Thus, this hybrid cell line constitutes a random genome approach to determining the background level of transcription in the human genome that is vastly greater in scale than anything previously attempted. We find that transcription in the human and Arabidopsis regions of the hybrid is similarly distributed, meaning we cannot exclude that pervasive transcription in the human genome is largely the consequence of background noise. This hybrid cell is a window into the nature of transcription in the human genome and will enable us to determine the sequence signatures that underlie pervasive transcription.



Local trade-offs shape flower size evolution across *Arabidopsis thaliana* distribution.

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MEETING ROOM 132, August 18, 2025, 2:00 PM - 5:00 PM

The evolution of flowers, largely driven by mutualistic interactions with animal pollinators, resulted in a vast diversity of shape and size that was believed to have spurred the intense evolutionary radiation in angiosperms. In this study, we investigate the geographic variation in reproductive organ growth in the self-fertilizing species *Arabidopsis thaliana*, where the absence of pollinator requirement for reproduction is expected to promote the reduction of flower organ sizes to optimize resource allocation. We find large variation in flower size associated with a polygenic architecture in which derived alleles increase or decrease flower size, and exhibit signs of positive selection. The variation in the direction of

flower size evolution is associated with a clear biogeographic pattern emerging from environmentally driven fluctuations in the flower size-seed production relationship. Stronger purifying selection at climatic range margins favors smaller flowers, while relaxed environmental constraints in more suitable habitats allow for the fixation of potentially adaptive large-flower mutations. Such a geographical pattern extends to other life history traits, demonstrating how environmental heterogeneity, in interaction with resource allocation constraints, shapes evolutionary trajectories and phenotypic diversity.



Long-fuse evolution of carnivoran skeletal phenomes through the Cenozoic

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MEETING ROOM 132, August 18, 2025, 10:30 AM - 12:30 PM

Ecological opportunities arising from environmental changes are often hypothesized to promote phenotypic diversification across macroevolutionary time scales. While neontological analyses are often used to test this hypothesis, extant data only captures the time-averaged signal of surviving lineages. More nuanced tests require paired and longitudinal climatic and organismal data from the fossil record. Here, we developed the most comprehensive phenomic dataset of pan-carnivorans (i.e., Carnivoramorpha, which includes Carnivora; Hyaenodonta; and Oxyaenodonta) to test hypotheses that environmental changes during the past 56 million years influenced the tempo and mode of the cranial, appendicular, and axial skeletal systems. We quantified the skeletal phenome by measuring 103 linear trait measurements from 118 extant species and 81 extinct species (854 specimens from 17 natural history museums). We then fit a series of macroevolutionary models to explain pan-carnivoran skeletal phenome disparity. We found nearly equal support for two release and radiate models, where the evolution of skeletal phenomes transitions from an Ornstein–Uhlenbeck process to Brownian motion at the Eocene-Oligocene

Transition (EOT) and at the Mid-Miocene Climate Transition (MMCT). Disparity through time analyses further reveal that the EOT led to a decrease in subclade disparity whereas the MCCT led to an increase in subclade disparity compared to null expectations under Brownian motion. Together, our results suggest that climate and environmental changes during these time periods facilitated the diversification of crown carnivoran skeletal phenomes under a long fuse model. Specifically, EOT-induced extinction of coeval stem pan-carnivorans released crown carnivorans from a constrained adaptive zone, resulting in initial phenotypic diversification among carnivoran families from the Early Oligocene to Mid-Miocene. However, further radiation of skeletal phenomes within families did not occur for another 20 million years until after the MMCT, enabling crown carnivorans to exploit new ecological niches facilitated by changing environments of the late Miocene to Pleistocene. Overall, this work highlights the importance of analyzing data from extant and extinct species under a phylogenetic comparative framework to disentangle the multiple climatic events that contributed to the evolution and diversity of carnivoran skeletal phenomes.



Machine learning evaluation of evolutionary accelerated regions reveals genomic basis of adaptation in bats

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MEETING ROOM 116, August 19, 2025, 2:00 PM - 5:00 PM

Lineage-specific accelerated substitutions in conserved regions can drive adaptive evolution. However, linking these accelerated changes to the traits they influence remains a major challenge. In this study, we analyzed a comprehensive catalog of bat-accelerated regions (BARs) by systematically evaluating their functional relevance using chromatin profiles, predicted target genes, and associations with synteny breaks.

To investigate the phenotypic consequences of BARs in limb development, we trained an embryonic limb accessibility model using functional genomic data from four

mammalian species, including two bats. With this model, we predicted molecular phenotypes across more than 100 mammalian genomes and identified BARs that contain bat-specific regulatory changes relevant to limb development. Finally, we dissected the regulatory landscape of these candidates, uncovering bat-specific transcription factor binding sites, structural variations, and 3D chromatin organization changes.

Our results highlight candidate loci underlying bat wing evolution and provide a framework for studying accelerated regions in an evolutionary and functional context.



Mechanisms of Community Assembly through the lens of Phylogenetic Diversity: a Critical Reappraisal.

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Darwin was one of the first to hypothesize a connection between niche differentiation and competition and species relatedness, offering an appealing framework to disentangle the processes involved in community assembly based on their phylogenetic structure. Community assembly is, however, the result of several processes including potentially confounding factors associated with dispersal limitations and spatial effects, casting doubt about the application of phylogenetic diversity metrics to infer community assembly processes. We implemented a spatially-explicit model

involving limited dispersal, drift, trait-based selection and competition to simulate community composition under competing assembly processes in a landscape with contrasted habitat connectivity. Our results formalize the idea that habitat selection and limiting similarity leave a significant signature in the phylogenetic structure of communities. We identify the phylogenetic diversity metrics that best perform to retrieve community assembly processes but raise caution regarding their interpretation, which can be misleading due to spatial effects.



Mitochondrial introgression affects routine metabolic rates and growth rates in tadpoles

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Background: Mito-nuclear incompatibility in natural hybrids has gained attention for its potential effects on fitness and life history traits including metabolism. This study investigates how metabolic rate, growth rate, and larval development are affected by mitochondrial introgression from *Pelophylax lessonae* (nucleotype LL) into *Pelophylax ridibundus* (nucleotype RR). We hypothesized that RR tadpoles carrying L mtDNA (introgressed) would show altered metabolic rates compared to RR tadpoles with R mtDNA (non-introgressed), and that any metabolic differences would be reflected in their growth trajectories and developmental timing. This work provides insight into the role of mito-nuclear interactions in amphibian physiology and the fitness consequences of inter-specific mitochondrial introgression.

Materials and Methods: Tadpoles were reared at constant temperatures of 18°C, 20°C, 22°C, and 24°C between 2021 and 2023. In 2023, larvae from the 20°C and 24°C treatments were tested for metabolic rate at four assay temperatures (16°C, 20°C, 24°C, and 28°C). Oxygen consumption was recorded using a closed respirometry system to estimate routine metabolic rate. Growth rate was quantified as the change in body mass across successive developmental stages of tadpoles. We applied linear mixed-effects models to evaluate the effects of mitotype, body mass, rearing temperature, test temperature, and parental cross (random effect) on metabolic rate, and included developmental stage as a covariate in growth models. Descriptive statistics summarized the number of days spent in each larval stage and associated mortality.

Results: Metabolic rate scaled positively with body mass and decreased with rearing

temperature. A significant interaction between test temperature and mitotype indicated that introgressed tadpoles showed distinct temperature sensitivity. Although differences at individual test temperatures were not statistically significant, introgressed larvae exhibited lower metabolic rate at 16°C, similar rate at 20°C, and higher rates at 24°C and 28°C. Differences in metabolic rate between assay temperatures were significant for introgressed tadpoles. Growth trajectories were parallel between mitotypes, but introgressed tadpoles maintained lower overall body mass. The disparity at initial and final body mass at 18°C was greatest between introgressed and non introgressed tadpoles, and decreased as rearing temperature increased from 20°C to 24°C.

Conclusions: These findings suggest that mitochondrial introgression influences temperature-dependent metabolic phenotypes. The effect of rearing temperature highlights a cold adaptation mechanism, wherein individuals exposed to lower temperatures exhibit higher metabolic rates to enable quicker responses to environmental changes. Although metabolic differences did not reach statistical significance at individual temperatures, the observed patterns correspond with reduced larval body mass in introgressed tadpoles. Mortality rates, duration of larval phases, and overall survival probability did not differ between mitotypes, although introgressed tadpoles showed higher variability in these parameters. We provide evidence that mitochondrial introgression of L mtDNA into *P. ridibundus*, occurring in many natural frog populations, affects tadpole performance and may have temperature-dependent, eco-evolutionary consequences.



Morphological diversification of continental and insular fish radiations follow similar evolutionary trajectories

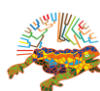
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Adaptive radiation, driven by ecological opportunity and release, can lead to rapid lineage and phenotypic diversification, contributing substantially to the ecomorphological diversity across the Tree of Life. Cichlid fishes, particularly those from insular systems such as the East African Great Lakes, are classic examples of this process. However, it remains unclear whether their morphological evolution follows similar trajectories across different landscapes, and how other fish radiations, spanning both continental and lacustrine systems, compare. To explore this, we examined multiple African fish radiations varying in biological characteristics, spatial scale, and ecological context. We generated detailed phenotypic datasets for three taxonomically independent continental-wide radiations: *Oreochromis* cichlids, *Synodontis* catfishes, and *Mastacembelus* spiny eels, in which the latter two groups have also radiated in the ancient rift lake, Tanganyika. As a comparison, we also

generated data on three well-studied lacustrine cichlid radiations from Lake Tanganyika that included the Lamprologini, Ectodini and Tropheini. We combined these datasets with published species-level phylogenies and applied phylogenetic comparative methods to investigate patterns and processes of morphological evolution. Despite contrasting spatial scales and life histories, our findings suggest that head and body shape diversification occurred under strong selective constraints. Surprisingly we do not observe an early burst model of morphological evolution for any group despite many of the lake species occurring sympatrically, and therefore interacting and coevolving while filling ecological space during their radiation. While our analyses support repeated morphological convergence in lake taxa, evident in their overlapping morphospace occupation, this process was less common in the continental radiations, where species typically occur in allopatry.



Natural variation in the gut microbiota is associated with behavioural differences in wild mice. But does it cause them?

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MEETING ROOM 118+119, August 19, 2025, 2:00 PM - 5:00 PM

All animals are home to microbial communities, the microbiota, yet microbiota composition varies widely among individuals within any population. The mammalian gut microbiota in particular has strong impacts on animal physiology, performing functions as varied as modulating immunity or aiding digestion. Recent studies suggest the gut microbiota also directly affects host behaviour, through a collection of mechanisms known as the microbiome-gut-brain axis. However, almost all research on microbiome-behaviour links to date has focused on lab animals and used highly artificial microbiota manipulations. It is thus unknown if natural variation in the mammalian gut microbiota can impact host behaviour in wild populations. Here, we address this knowledge gap by studying microbiota-behaviour links in a natural system, the wild house mouse population of Skokholm Island. Over 3 years, we quantified anxiety-like and exploratory behaviours of 225 mice, and in parallel characterised their gut microbiota. While no intrinsic (sex, morphometrics) or extrinsic (season, ambient temperature or light) factor explained the considerable variation found in

mouse behaviour, we find that microbiota dissimilarity metrics between mice are associated with differences in several behavioural traits. These results suggest that the microbiota is either a key player in shaping animal behavioural variation in the wild, or is itself affected by natural differences in animal behaviour. While these correlative results are interesting, to disentangle the causal direction of this microbiota-behaviour links in wild animals, we then conducted faecal microbiota transplant experiments, where we inoculated germ-free lab mice with microbiotas collected from behaviourally-phenotyped wild mice. The results of this first-of-a-kind experiment will also be presented here: if we find that behavioural phenotypes in recipients match those of donors, it will be convincing evidence for natural variation in microbiotas contributing to wild animal behaviour differences. If recipient behaviour does not recapitulate donor behaviour, it will instead suggest that our correlative associations between animal behaviour and the microbiota may instead reflect host behaviour shaping microbiota composition in wild animals.



Non-hypermulator cancers access driver mutations through reversals in germline mutational bias

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MEETING ROOM 116, August 19, 2025, 11:00 AM - 1:00 PM

Cancer is an evolutionary disease driven by mutations in asexually-reproducing somatic cells. In asexual microbes, bias reversals in the mutation spectrum can speed adaptation by increasing access to previously undersampled beneficial mutations. By analyzing tumors from 20 tissues, along with normal tissue and the germline, we demonstrate this effect in cancer. Non-hypermuted tumors reverse the germline mutation bias and have consistent spectra across tissues. These

spectra changes carry the signature of hypoxia, and they facilitate positive selection in cancer genes. Hypermuted and non-hypermuted tumors thus acquire driver mutations differently: hypermutated tumors by higher mutation rates and non-hypermuted tumors by changing the mutation spectrum to reverse the germline mutation bias.



Not the same: genomic analysis explains variation in strength of positive selection across avian immune genes

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The emergence and spread of infectious and zoonotic diseases, combined with ongoing habitat fragmentation and loss of genetic diversity, pose a significant threat to global health. Understanding the evolutionary dynamics of immune systems in wild animal populations is, therefore, crucial for anticipating and mitigating future disease outbreaks. However, this is all complicated by the fact that the vertebrate immune system is extraordinarily complex, comprising more than 2,000 genes with immune function, and posing a significant challenge: how can we identify disease resistance genes, akin to searching for needles in a haystack?

One can hypothesise that genes that are in the first line of immune defence and/or physically interact with pathogen molecules are subject to stronger diversifying selection. However, current knowledge of immune gene evolution is only fragmentary and largely limited to a few well-studied immune genes, especially those of the major histocompatibility complex genes (MHC) and pattern-recognition receptors (PRRs). Consequently, we lack knowledge about broader trends in the evolution of immune genes. Does the strength of positive selection differ depending on the molecular function or cellular localisation of the gene?

To answer these questions, we conducted the largest comparative analysis of positive selection in immune genes to date, using 50 publicly available bird genomes. Birds provide an exceptional model for studying the adaptive evolution of immune genes across vertebrates, having evolved in parallel with mammals since their last common ancestor over 310 MYA, serving as reservoirs for multiple zoonotic pathogens.

Building on our recent review on evolutionary immunology (Vinkler et al. JEB 2023), we first compiled a novel list of 453 immune genes with resolved orthology between birds and mammals, including only those with significant roles in

immunity. Using our newly defined Immunome concept, we then semi-manually classified these genes into five novel distinct immune catalogues reflecting their functions and cellular locations.

Applying three codon-based dN/dS methods, we revealed positive selection in 363 protein-coding immune genes, of which 36 genes were under strong selection (>5 % codons under selection). Surprisingly, we found no difference in intensity of selection between ancient innate and more derived adaptive immunity, likely due to the functional integrity of both branches of immunity, which form interlinked layers of regulatory and effector mechanisms. Importantly, core immune genes (i.e. those with primary functional role in immunity) were subject to stronger selection than peripheral immunome genes (i.e. those having multiple functions including non-immune ones). Effector molecules showed stronger selection than genes with signalling, enzymatic or recognition functions. Membrane-bound and extracellularly secreted immune genes underwent stronger positive selection than those expressed in cytosol or nucleus. Among them, the most strongly selected categories were antimicrobial proteins, complement and PRRs. Comparing our functional classification with standard gene overrepresentation analysis showed that our approach better characterises major trends in immune gene evolution. These results suggest that immune genes with primary roles in immunity and those whose products physically interact with pathogen structures are subject to stronger positive selection than other immune genes. Such categories and gene candidates under strong adaptive evolution should be prioritized as targets in future interspecific immunogenetic research — e.g., to test their associations with microbial or viral communities, disease phenotypes, or to support applications in animal breeding and conservation genetics.



Novel genomic approaches support Xenacoelomorpha as sister to all Bilateria

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MEETING ROOM 117, August 21, 2025, 2:00 PM - 5:00 PM

One of the most contentious debates in animal evolution is whether the first bilaterian animal was simple or complex. At the centre of this debate is the phylogenetic placement of the enigmatic lineage known as Xenacoelomorpha. For the last 25 years, different phylogenies have shown Xenacoelomorpha to be either a sister group to the rest of the bilaterians or inside the deuterostomes, with some analyses challenging the monophyly of deuterostomes. To solve this conundrum, we mined thousands of gene families from 91 complete genomes and analysed them using various alignment-free genome-wide evolutionary processes. We also applied

these approaches to inform the construction of a novel metazoan gene matrix 15 times larger than previous datasets. This dataset was also used to reconstruct the protein complement present in the genome of the last common ancestor of bilaterians. These datasets were thoroughly analysed with approaches and evolutionary models to reduce systematic artifacts. Our results strongly support Xenacoelomorpha as the sister group to all other bilaterians and the monophyly of deuterostomes, pointing to a simple bilaterian, while illustrating the phylogenetic potential of genomic-level approaches.



On the evolution and mechanisms of reproductive diapause in tropical insects

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There are seasonal patterns in ecosystems across the world, and species that experience these have evolved a range of plastic strategies that allow them to maintain fitness in seasonally varying habitats. One key adaptation in insects is reproductive diapause, where adult females halt oogenesis and mating behaviour in the unfavourable season. This plastic trait allows individuals to 'wait out' poor environmental conditions, enabling occupancy of a wider range of seasonal environments compared to individuals or species without diapause.

Comparatively little is known about reproductive diapause in the tropics, despite many tropical ecosystems exhibiting strong seasonality. It has been hypothesised that butterfly diapause originated in the tropics, but with one notable caveat: very little is known about insect diapause in the tropics. Key open questions on tropical diapause include how seasonality affects reproductive behaviour, what environmental cues drive this, what genetic mechanisms underlie such plasticity, and how diapause evolves.

Here, we aim to test the hypothesis that diapause originated in the tropics and determine how these adaptations have evolved across the Nymphalid phylogeny. We will then use transcriptomic information across tissues to test whether a global 'diapause toolbox' exists. Finally, we aim to understand the role that the climate plays in producing and maintaining these traits.

We studied seasonal dynamics of butterfly reproduction in the Amazon rainforest. The Amazon is an important biodiversity hotspot and a major geographic origin of butterflies. Parts of it experience a large change in climate throughout the year with a pronounced annual dry season. First, we analysed seasonal reproductive dynamics across a phylogenetically broad range of neotropical butterfly species in the Nymphalid

family to understand the origins of diapause. We found a range of reproductive dynamics, even within genera, leading us to then focus on two closely related species: *Catonephele acontius* and *C. numilia*. Here, we found significantly different reproductive dynamics, with *C. acontius* undergoing a pronounced diapause during the dry season, whilst *C. numilia* does not. These results indicate recent divergent evolution that may have happened through the evolution of suites of correlated plastic traits.

Next, to identify the genetic mechanisms of diapause and compare with temperate insects, we analyse transcriptomes in the brain, fat body and ovary of diapausing and non-diapausing individuals in dry and wet seasons. We first assembled and annotated long-read, high-quality genomes of both target species, and we are now uncovering the transcriptomic pathways involved in producing a diapause response.

Finally, we analysed how the reproductive dynamics of *C. acontius* are associated with the climate to predict responses to future climate change. We found that maximum temperature is the main driver, likely via a threshold that varies within the population. We hypothesise that other climatic factors (including photoperiod) are responsible for setting the temporal boundaries of the direct temperature responses.

Much less is known about the impact of climate on seasonal behaviour in tropical insects than temperate ones, and our work contributes to this knowledge using long-term field data. Our work supports a tropical origin of diapause because of the variation that we see across the Nymphalid family and sheds light on the evolutionary paths taken to create such variation. Our climate analysis suggests that these traits will respond quickly to a warming climate and may allow insects in seasonal environments to thrive in times of rapid climate change.



Opsin gene expression plasticity and spectral sensitivity in male damselflies could mediate female colour morph detection

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MEETING ROOM 132, August 18, 2025, 10:30 AM - 12:30 PM

The visual systems of Odonata (dragonflies and damselflies) are characterized by many opsin genes, which form the primary light-sensitive photopigments of the eye. Female-limited colour polymorphisms are also common in Odonata, with one morph typically exhibiting male-like (androchrome) colouration and one or two morphs exhibiting female-specific colouration (gynochromes). These colour polymorphisms are thought to be maintained by male mating harassment and frequency-dependent sexual conflict, in which males form search images for certain morphs, causing disproportionate mating harassment. Here, we investigate opsin sensitivity and expression plasticity in the damselfly *Ischnura elegans* over adult maturation and in populations with different female morph frequencies. We find evidence for opsin-specific plasticity in relative and

proportion opsin mRNA expression, suggesting changes in opsin regulation and visual sensitivity over adult maturation. In particular, expression of the long-wavelength sensitive opsin LWF2 changed across adult development and between populations exhibiting directional changes in female morph frequencies. UV-Vis analyses indicate that short- and long-wavelength opsins absorb wavelength light between 350 and 650 nm. Assuming opponency between photoreceptors with distinct spectral sensitivities, measured sensitivities can confer spectral fine visual discrimination between androchrome and gynochrome females. Overall, our results suggest that opsin sensitivity and expression changes could contribute to visual tuning impacting males' ability to detect and discriminate between female morphs.



Parallel gene losses underly convergent evolution of the oral apparatus in ant-eating mammals

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MEETING ROOM 116, August 19, 2025, 11:00 AM - 1:00 PM

Background: Adaptation to ant and/or termite consumption (myrmecophagy) in mammals constitutes a textbook example of convergent evolution, being independently derived in several mammalian lineages. Myrmecophagous species are characterized by striking convergent morphological adaptations such as skull elongation, enlargement of salivary glands, and long claws to dig into ant and termite nests. These evolutionary modifications also include anatomical regression, such as dental simplification or loss, reduction of masticatory muscles, and possessing a reduced set of taste buds.

Materials and methods: To gain insights into the molecular changes underlying the regression of these morpho-anatomical traits, we investigated the functionality of candidate genes related to dentition, gustation, and mastication in nine convergent myrmecophagous mammalian lineages. We examined these genes in a comparative phylogenetic context, paired

with molecular evolutionary analyses, to estimate the relative timing of loss of gene function over the evolutionary history of each convergent lineage.

Results: We found that gustatory reduction and pseudogenization of masticatory myosin often were associated with the regression of dental genes. Evidence of pseudogenization events linked to oral anatomy dates to as early as the Cretaceous/Paleogene boundary, and is an ongoing process including examples of incipient gene inactivations. Whereas we found evidence for gene inactivations across all three functional categories occurring during distinct temporal intervals, there was variation in the sets of genes lost and the relative timing of inactivation events.

Conclusions: The combined evidence suggests that the convergent evolution of myrmecophagy has occurred as a protracted process with distinct phases of anatomical evolution, over timescales as long as 60 Myr.



Patterns of Duplicate Gene Retention Over Different Timescales and With Different Selective Pressures

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MEETING ROOM 116, August 21, 2025, 2:00 PM - 5:00 PM

Gene duplication is an important process leading to gene content evolution in genomes. Three classes of models for gene duplicates will be described. The first model uses a Moran modeling framework to examine gene duplicates that are segregating in a population. Based upon the age of the duplicate and the frequency in the population, a test is performed to ask if the frequency is consistent with neutral processes or if there is evidence for selection on the duplicate itself.

A second modeling framework examines the interplay between dosage balance and functional evolution through subfunctionalization. The model suggests that from whole genome duplication events, dosage balance presents a selective barrier that must be overcome, but ultimately leads to enhanced retention of duplicates. For smaller scale duplicates, the reverse is true.

Lastly, it has been observed that some genes are more likely to be retained after duplication than other genes from a whole genome duplication event. A formal model based upon organismal gene content is presented that provides a time-dependent expectation for duplicate gene retention when different combinations of processes are acting. This modeling framework has been applied to sets of whole genome duplication events in fish and plants, characterizing both the gene contents and preservation processes that have acted.

Together these modeling frameworks provide community tools and present a picture of the processes acting on gene duplicates at different stages.



Phenotypic and genotypic evidence of physiological and ecological costs of tomato defence suppression in the spider mite *Tetranychus evansi*

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MEETING ROOM 132, August 18, 2025, 2:00 PM - 5:00 PM

Herbivores have evolved a plethora of strategies to cope with plant defences. Possibly, the maintenance of such diversity results from associated costs, a hypothesis lacking empirical validation. Defence suppression is a particularly enthralling counter-defence strategy, as resources made available by suppressors can also be exploited by potential competitors. This suggests that, besides physiological costs, ecological costs may explain why such strategy is not pervasive among herbivores. Here, we perform the first empirical test to the existence of such costs in *Tetranychus evansi*, a herbivorous spider mite known to suppress tomato defences. To this aim, we performed 26 generations of experimental evolution of spider mite populations on tomato plants with impaired induced defences (a) or with intact induced defences in absence (b) or presence of competitors (c). Defence suppression was counter-selected in environments where it did not

yield a benefit (regime a) and in which this benefit could be reaped by competitors (regime c), confirming the existence of physiological and ecological costs, respectively. Importantly, fecundity on plants with intact defences decreased, but only in populations evolving with competitors, suggesting that distinct mechanisms underlie physiological and ecological costs of defence suppression. This set of phenotypic results was confirmed at the genotypic level, with over 100 genes being overexpressed in the regimes in which suppression was counter-selected compared to the control regime, including some genes related with proteolysis and transmembrane transport. Such a fast response to the imposed selection pressures reveals the existence of variability in defence suppression in the ancestral mite population, a key ingredient to explain the heterogeneity in coping with plant defences observed among herbivores.



Phenotypic Plasticity in Predator-Induced Defenses: Species- and Genus-Level Responses to Invertebrate Predation in Cladocerans

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MEETING ROOM 118+119, August 19, 2025, 2:00 PM - 5:00 PM

Predation is a fundamental evolutionary force shaping the development of antipredator traits and structuring ecological communities. Phenotypic plasticity, particularly in the form of predator-induced defenses, allows prey species to respond adaptively to varying predation pressures.

Here, we examined the extent of such plasticity across multiple cladoceran taxa in Lake Constance using both experimental and long-term observational approaches. In a common garden experiment, we exposed clonal lineages of three *Daphnia* species (*D. cucullata*, *D. galeata*, *D. longispina*) and two *Bosmina* species (*B. coregoni* and *B. longirostris*) to predator kairomones from *Bythotrephes longimanus* and *Leptodora kindtii* for ten days. To assess long-term dynamics, we also analyzed three decades of data on *Bosmina* species under changing top-down (fish predation) and bottom-up (nutrient availability) pressures in the lake.

Daphnia species exhibited species-specific plastic responses. *D. cucullata* and *D. galeata* developed distinct morphological defenses under predator exposure, while *D.*

longispina showed negligible change, likely due to reliance on behavioral defenses such as diel vertical migration. In contrast, *Bosmina* species demonstrated consistent plastic changes, including elongation of antennules and mucro, and increased eye diameter. These responses were similar between the two *Bosmina* species. However, the presence of transgenerational maternal effects suggests a complex, stage-dependent expression of plasticity in response to predation. Long-term data revealed stable species composition and traits during the first two decades of nutrient decline, the final decade was marked by rapid shifts in both. These shifts likely reflect intensified fish predation and cascading effects on invertebrate predator-prey dynamics. Notably, trait changes at the genus level did not fully reflect species-specific responses, underscoring hidden diversity in plasticity within genera.

Our results demonstrate that phenotypic plasticity plays a key role in mediating predator-prey interactions and shaping community composition in the lake.



Phylogenetic inference with not-so-rare mutations and wee tiny organisms

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MEETING ROOM 117, August 21, 2025, 2:00 PM - 5:00 PM

A common assumption in molecular evolution is that mutations are rare. This assumption is widely used in the development of evolutionary theory, an example being the infinite sites model, which posits that the genome has infinitely many sites, such that each new mutation necessarily occurs at a unique site. Rare mutations are a simplifying assumption, and it is well established that recurrent mutations - particularly in highly diverse organisms such as bacteria and viruses - occur frequently.

In this study, we challenge the assumption of rare mutations in phylogenetic inference. In a recent study, we demonstrated that ignoring recurrent mutations leads to inflated estimates of effective population size. We therefore expect that unaccounted recurrent mutations influence the rate of

molecular evolution and, consequently, distort phylogenetic inference.

To investigate this, we developed two models of evolution: one that incorporates recurrent mutations, and another that allows only boundary mutations (akin to the infinite sites model). We generated multiple sequence alignments using the former and inferred phylogenies using the latter. While the inferred tree topologies were correctly inferred across models, the estimated branch lengths and mutation rates were substantially biased. We also show that standard nucleotide substitution models fail to accurately estimate phylogenies, although it appears that the correct topology may still be recovered given sufficiently large data. Finally, we discuss the implications of these findings for phylogenetic inference, drawing on microbial case studies of epidemiological and medical relevance.



Plasticity and evolution of metabolic division of labour within families

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MEETING ROOM 118+119, August 19, 2025, 2:00 PM - 5:00 PM

In many species parents transfer materials, such as milk or regurgitate, to their developing young. The primary function of these is to provide nutrition or symbionts. However, these socially transferred materials also transmit a complex array of secondary molecules like proteins, RNAs and hormones which can assist offspring with growth, immunity and digestion. Therefore, these transfers could allow the metabolic burden of development to be shared between parents and offspring.

Traditionally, parental care behaviours have been characterised as acts of altruism, in which parents work to promote the growth and fitness of their helpless offspring at some cost to their own future fitness, and thus the focus is on the parents and the labour they undertake when supplying care. However, this characterisation excludes the contributions that offspring make to their own development. We reframe parental care behaviours, such as social fluid exchange, as a division of labour between parents and offspring, with both parties working towards the common goal of offspring achieving independence and full responsibility for their growth, development and survival. This allows us to more accurately measure the plasticity and co-evolution of parental care related traits in both parents and offspring, and more easily quantify the costs and benefits of these behaviours using molecular techniques. Additionally, by reframing the effort divided between parents and offspring as a division of labour we can deepen the perspective of parental care towards a more functional viewpoint, and integrate theory from the wider division of labour literature.

Under this framework, we used experimental evolution to test 1) whether the balance of metabolic division of labour changes

evolutionarily in both parents and offspring when parents are experimentally prevented from providing metabolic assistance, and thus whether social transfers entrench interdependencies between parents and offspring and 2) whether there is plasticity in the extent of metabolic division of labour, which offers capacity for greater offspring independence and provides a mechanism for offspring to escape obligate dependence on care. In the burying beetle *Nicrophorus vespilloides* parents deposit oral fluids onto their carrion nest during pre-hatching care, and facultatively transfer fluids to larvae through regurgitation as post-hatching care. We analysed the oral fluid proteomes of replicate experimental populations that had been evolving for 50 generations with or without post-hatching care, and which were then allowed to produce larvae with or without post-hatching care for one experimental generation. We found that parents and larvae plastically and evolutionarily adjusted the endogenously produced proteins in their oral fluids. Regardless of their evolutionary history, larvae that were deprived of parental care for one experimental generation upregulated oral fluid proteins with putative functions relating to digestion, immunity and growth. However, under these conditions, larvae that had evolved with post-hatching care were less capable of consuming carrion proteins than larvae that had evolved without post-hatching care, and had lower survival. This suggests that larvae evolving without care were less obligately dependent on care than their counterparts that were evolving with care. Our results suggest that metabolic division of labour within families is largely plastically modulated, but that the extent of plasticity can evolve rapidly when social conditions change.



Polarizing SNPs without outgroup

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MEETING ROOM 116, August 21, 2025, 2:00 PM - 5:00 PM

Determining whether an allele at a polymorphic site is ancestral or derived, a process known as polarization, provides essential prior knowledge for various analyses in population and quantitative genetics. For instance, it can be used to detect adaptive evolution, introgression, estimate allele age and the strength of local selection. Furthermore, it also enables the calculation of the unfolded site frequency spectrum (uSFS), which captures the distribution of derived allele frequencies across the genome and contains rich information about demographic history, patterns of gene flow, and selection. Currently, the most widely used approach for polarization relies on data from outgroup species. However, in many cases, particularly for species lacking a closely related outgroup, this strategy can result in substantial information loss due to species divergence.

Here, we develop PolarBEAR (Polarization By Estimation of the Ancestral Recombination graph), an outgroup-free method that infers ancestral states and estimates uSFS using the local genealogies from the ancestral recombination graph (ARG) and based on an empirical Bayesian approach.

As a theoretical basis, we first explore under which conditions the local genealogies in ARG contain information about the ancestral state. Then we show that PolarBEAR reaches high accuracy in polarization and uSFS estimation using simulations under several

scenarios. This accuracy, however, depends strongly on the quality of the input ARG: performance is high in the theoretical situation where the ARG is known, but can vary substantially when ARGs are reconstructed from the data with different inference methods. We further applied PolarBEAR to human population genomic data and compared its results to those obtained using est-sfs, an established outgroup-based polarization method. Although PolarBEAR could polarize fewer sites than est-sfs on our benchmark dataset due to the difficulty in inferring ancestral states with high confidence at sites with certain local genealogies, it polarized positions that est-sfs could not handle due to missing outgroup data. At positions inferred by both methods, the inferred ancestral states showed high concordance. The two methods inferred similar uSFS, with PolarBEAR estimating slightly fewer high-frequency derived alleles. Furthermore, we show that PolarBEAR is relatively robust to model misspecification, as it does not rely on an outgroup and thus avoids biases in the uSFS estimates introduced by assumptions about the substitution process, while est-sfs exhibits a bias in the presence of heterogeneous base composition. PolarBEAR can complement outgroup-based methods, or be employed as a substitute when no appropriate outgroup sequence is available.



Population genomics resolves the complex domestication origin of adzuki bean

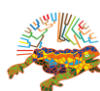
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MEETING ROOM 116, August 21, 2025, 2:00 PM - 5:00 PM

The adzuki bean (*Vigna angularis*) plays an important role in East Asian culinary culture. Previous genetics studies suggested the highest cultigen genetic diversity in Central China, suggesting a domestication origin there. Archaeological evidence, on the other hand, identified the earliest traces of cultivation in Japan, associated with the Jomon culture. From a comprehensive collection of about 700 diverse wild and cultigen accessions across Asia, here we

used population genomics to resolve its complex domestication origin. We further performed genetic mapping, identified the genes associated with the change in seed coat color, and validated the gene functions in planta. By modeling the evolution of these mutants, we unraveled a unique evolutionary process of domestication syndrome, supporting recent viewpoints of the early selection before large-scale wild-cultigen genomic divergence.



Reconstructing the Evolution of Iridescence Across Birds

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MEETING ROOM 132, August 18, 2025, 10:30 AM - 12:30 PM

Some of the most striking colors in nature are found in the iridescent feathers of birds, such as peacocks. These colors arise from the nanoscale organization of melanin-containing organelles, producing angle-dependent iridescence that ranges from weak (as in swifts) to brilliant (as in peacocks). Despite their prominence as textbook examples of sexual and natural selection, the evolutionary trajectories of iridescence remain unclear.

Here, we assembled a large database quantifying iridescence intensity from weak (i.e. swift-like) to brilliant (i.e. peacock-like) for 71,536 body patches from 5,755 species, representing all avian families. We used this dataset to reconstruct the evolution of

iridescence across bird species and body. Our findings suggest that ancestral birds—and likely even dinosaurs—exhibited brilliant iridescent colors comparable to those seen in peacocks today. While iridescence was ancestral it was repeatedly lost throughout the evolutionary history of birds, but re-evolved multiple times, with a gradual transition from weak to brilliant iridescence. Furthermore, iridescence evolved from both melanized and non-melanized patches. Finally, the uneven distribution of iridescence between sexes and body regions suggest a function in signalling in both birds and their ancestors. Indeed, the only consistent predictor of increased iridescence intensity is sexual selection.



Repeated emergence of specialised ribosomes suggests a complex role for deterministic evolutionary forces across eukaryotes

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MEETING ROOM 116, August 19, 2025, 2:00 PM - 5:00 PM

Ribosomes are essential to all cellular life but not all eukaryotic ribosomes, even within the same cell, are equivalent. Instead, subtle compositional differences between ribosomes, including differential inclusion of ribosomal protein paralogs, have been shown to significantly change the translational output of the ribosome, in a process known as ribosome specialisation. We now have evidence of ribosome specialisation across all major groups of eukaryotes allowing us to address fundamental questions in evolutionary biology about convergent evolutionary processes. By studying repeated emergence of ribosome specialisation across these vast evolutionary distances, we can ask the extent to which molecular evolution at the level of highly functionally constrained protein complexes is deterministic or contingent. We use a phylogenetic, gene tree-species tree reconciliation approach to test these competing hypotheses in the context of specialised ribosomes. Our results suggest that the evolution of the

ribosome is highly dynamic in structure, with ribosomal gene duplication and divergence acting at high rates throughout the tree of eukaryotes. Further, we find that known cases of specialisation tend to occur through duplication of the same genes in different lineages, exhibiting convergent instances of evolution and suggesting a deterministic process. Finally, we outline the ways in which the evolution of specialisation can be generalised. We show that pathways to specialisation through paralog formation is not enriched in any particular part of the 3D ribosome and is not associated with a shift in selective pressure in paralogous proteins, and that these gene duplication events are rarely enriched in a given time period (with the exception of some plant whole genome duplication events). We conclude that the evolution of specialised ribosomes paints a complex picture of deterministic evolutionary forces which ought to inform our understanding of the evolution of a diverse range of protein complexes.



Resampling multiple genomic matrices to detect challenging nodes

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MEETING ROOM 117, August 21, 2025, 2:00 PM - 5:00 PM

Currently, complete genome assemblies are available for thousands of species making the vision of a resolved tree of life based on molecular data a practical reality. However, there are still several remaining difficult ancient nodes to resolve, such as the phylogeny of chelicerates. That can be partially caused because the analyses of genome scale datasets are often compromised by the computational burden of large phylogenomic analyses.

We propose a workflow to assess the robustness of phylogenetic results using multiple multi-protein, clade-specific matrices with different taxa and different genes. In each matrix, we include different species for each of the major clades within our group of interest, we infer single-copy orthologs, and analyse the resulting concatenated supermatrices using complex

models. We assess the nodal support for competing topologies under different strategies, such as outgroup removal and recoding, both at the aminoacid and nucleotide level. We applied this approach for the internal nodes of 3 different ancient groups: chelicerates, mollusks and vertebrates.

We found that while the phylogenies of mollusks and vertebrates show stable support across strategies, chelicerate phylogenies are greatly condition-dependant. Nonetheless, when topology tests were applied to assess the robustness of alternative topologies (arachnid monophyly/paraphyly) neither of them could be rejected. This suggests that the evolutionary history of chelicerates is still far from resolved, even with all the genomic data currently available.



Resolving Difficult Nodes in the Bat Phylogeny Using 103 Chromosome-Level Genomes

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MEETING ROOM 117, August 21, 2025, 2:00 PM - 5:00 PM

Despite being one of the most iconic mammalian radiations, the phylogeny of bats (Chiroptera) has remained unresolved at several deep and shallow nodes. As the only mammals capable of powered flight and with unique adaptations such as special immunity, echolocation, and extreme longevity, bats present an ideal system to explore both the biological and analytical challenges that hamper phylogenomic resolution across the Tree of Life. The Bat1K consortium generated and analyzed 103 chromosome-level long-read genomes representing all 21 bat families. This unprecedented dataset—both in taxonomic breadth and genomic quality—enabled a comprehensive re-evaluation of long-debated relationships at the suborder, superfamily, and family levels. We leveraged a diverse array of molecular characters, including annotations of >17K coding genes, neutrally evolving regions, and several whole genome alignments, both reference-based and reference-free. By integrating and comparing all these datasets, we aimed to

minimize alignment bias and orthology detection error, known contributors to gene tree discordance. Our analyses represent a spectrum of strategies from coalescent-based species tree methods and concatenation to topology weighting, sliding-window analyses, and gene-wise signal exploration, allowing us to assess the impact of modeling assumptions and locus selection across genomic scales.

We present a new, strongly supported phylogeny placing key lineages at different positions.

Together, our study demonstrates how densely sampled, chromosome-scale genomes, paired with novel and integrative analytical frameworks, can illuminate the most challenging phylogenetic questions and solve controversial nodes, particularly those associated with rapid radiations. Beyond bats, our approach is an example for tackling recalcitrant nodes across the tree of life, highlighting the need to embrace data heterogeneity and the inclusion of diverse molecular signals in the post-phylogenomic era.



Resurrecting a 100-year-old hypothesis: Effects of sheltering on sex chromosome degeneration and sex-biased gene content evolution

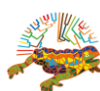
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MEETING ROOM 131, August 19, 2025, 2:00 PM - 5:00 PM

Sex chromosome systems have arisen many times, independently, yet they exhibit many common features. Y chromosomes often evolve extensive gene losses and become enriched for genes important for male fertility, whereas X chromosomes evolve modest gene losses and, in some cases, become enriched for genes primarily expressed by females. These features are thought to reflect an array of evolutionary mechanisms that include selective interference between non-recombining Y-linked variants, gene expression coevolution between sex chromosomes, and interchromosomal gene traffic. Here, we argue that these features are also consistent with a simple model of ‘sheltering’. Sheltering, as originally proposed by Muller (1914), refers to the evolutionary accumulation of recessive loss-of-function (LOF) mutations in Y-linked regions that are permanently heterozygous with the X. This

hypothesis was once popular but fell out of favour in the 1970s because early mathematical models failed to support Muller’s intuition and available data on dominance and dosage compensation were viewed as incompatible with the hypothesis. We reconsider the major arguments against Muller’s model, given modern data on dominance and dosage compensation, and develop an extended model of sheltering that relaxes restrictive assumptions of earlier models. Our results suggest that sheltering might contribute substantially to sex chromosome evolution, including commonly observed patterns of sex-linked gene losses and enrichments for female- and male-biased genes on the X and Y. Sex chromosomes are undoubtedly influenced by a wide range of evolutionary factors. We argue that sheltering should be seriously considered as a potentially important one.



See you in the dark: molecular evolution of extraordinary vision in deep-sea fishes

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MEETING ROOM 116, August 19, 2025, 2:00 PM - 5:00 PM

Fishes living in extreme environments such as the deep ocean have evolved unique adaptations to cope. It is mainly the absence of light and a limited color spectrum that have driven the evolution of numerous visual system adaptations in deep-sea fishes. Here, we focus on the molecular adaptations of deep-sea fishes using genomics and transcriptomics to explore genes essential for vision. Our primary focus is on rhodopsins, the light-sensitive pigments in rod cells that enable vision in dim environments. While most fishes possess one copy of the rhodopsin gene, some deep-sea species have evolved an exceptional diversity of rhodopsins through multiple gene duplications. Spinyfins (Diretmidae; Trachichthyiformes) represent an extreme example of visual adaptation, possessing multiple rod opsins (up to 38) that are sensitive to the blue-green light spectrum, ranging from 444 to 519 nm. Additionally, several of their rhodopsins have undergone extreme functional modifications, resulting in some of the shortest-wavelength-sensitive rhodopsins known in vertebrates. Furthermore, the binding sites for the G-protein transducin (which processes the

signal further along the cascade) are mutated in about half of the genes, possibly indicating a major functional change or even a switch in the type of G-protein used—a feature that, if confirmed, would be perhaps unique among vertebrates. Despite the high proportion of repetitive content in the silver spinyfin's genome, we inspected an assembled almost chromosome-level genome. We report that these multiple rhodopsin genes are arranged in two (or possibly three) genomic clusters, which are also functionally organized—that is, blue-shifted rhodopsins with mutated transducin-binding sites cluster together, while green-shifted rhodopsins are located in a separate cluster. Interestingly, deep-sea fish larvae inhabit shallow waters, where the light conditions differ significantly from the deep sea. As a result, deep-sea fish have evolved adaptations that accommodate the distinct visual demands of both life stages—the shallow-water larva and the deep-water adult. This phenomenon is particularly intriguing in spinyfin larvae, making them an exciting case for studying visual system evolution.



Sex chromosome turnovers in fish

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MEETING ROOM 131, August 19, 2025, 2:00 PM - 5:00 PM

Sex determination is highly variable and can be driven by different environmental and genetic factors. Genetic sex determination includes many different types of sex chromosomes. While the detection of sex chromosomes has seen a tremendous uprise with the advance of sequencing data, our knowledge on driving forces and mechanisms of sex chromosome emergence and turnover is limited. Changes in sex determination systems are particularly frequent in fish, the main model system of our research. Within the hyperdiverse fish family Cichlidae we detected an unprecedented high rate of sex chromosome turnover involving different chromosomes and yet non-random sex chromosome recruitment. We demonstrate cases of sex chromosome turnover with and without changes in heterogamety. We show that in addition to inter- also intraspecies variability

exists in sex chromosome constellations and that sex-bias in gene expression rapidly evolves on new sex chromosomes in different ways in XY and ZW systems. We find little evidence for sexual antagonism driving sex chromosome emergence. Novel sex chromosomes are often formed through chromosomal fusion suggesting that large-scale genomic changes are coupled to sex determination and ultimately speciation in cichlids. In a second fish family, Syngnathidae (seahorses and pipefish), we show that sex chromosomes are also not conserved between and again even within species. In syngnathids, sexual antagonism evidenced by the evolution of sex-specific gene expression and sex differences in allele frequencies is impacted by the combination of precopulatory sexual selection and male pregnancy. In summary, our research sheds light on the causes and consequences of sex chromosome turnover.



Single-Cell Dissection of Evolved Malnutrition Adaptation in *Drosophila* Larval Fat Body

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MEETING ROOM 132, August 18, 2025, 2:00 PM - 5:00 PM

Chronic nutrient limitation drives profound physiological and developmental adaptations, yet the cellular basis of these changes remains poorly understood. We have leveraged a *Drosophila melanogaster* experimental evolution model in which ten populations—five “Selected” lines reared for >300 generations on a nutrition-limiting poor diet and five “Control” lines maintained on standard nutrient levels—exhibit heritable differences in growth rate, adult body size, fat storage, and amino-acid assimilation. Bulk transcriptomic profiling of whole larvae identified metabolic pathways associated with these adaptive traits, but lacked the resolution to assign changes to specific cell types and provide clear direction of these metabolic shifts.

To bridge this gap, we have undertaken single-cell RNA sequencing of the larval fat body, the primary organ for energy storage, nutrient sensing, and inter-organ signaling during development. By profiling tens of thousands of nuclei from third-instar fat bodies of Selected and Control populations under the same nutritional regime, we chart the full repertoire of fat-body cell states at a critical developmental checkpoint and determine how long-term selection

reconfigures their gene-regulatory programs to aid adaptation at the cellular transcriptomic level.

We compare fat-body cell compositions to identify subpopulations that expand, contract, or emerge uniquely in Selected versus Control lines. We examine single-cell transcriptional profiles to see how metabolic modules—lipid handling, amino-acid transport, energy sensing, and signaling—are remodeled by long-term selection. Finally, we are looking at evidence of adaptive trade-offs, that accompany enhanced growth under nutrient limitations but a trade-off in development itself.

By integrating cell-type maps with different evolutionary regimes, we identify the genes and pathways mediating these evolved nutritional phenotypes. This single-cell atlas offers novel insights into how selection pressure on whole-animal performance translates into re-wiring of the specific cellular architectures and networks. Our findings will illuminate the cellular logic of quantitative metabolic adaptation and establish a generalizable framework for linking organismal evolution to cell-type-specific transcriptomic shifts under environmental stress.



Sperm - female reproductive fluid interactions in a fish with a parasitic reproductive strategy

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MEETING ROOM 115, August 21, 2025, 2:00 PM - 5:00 PM

Female reproductive fluids (FRF hereafter) are media produced by females through which sperm must pass on the way to fertilise the eggs. Accumulating evidence indicates that they can influence the outcome of gamete interactions in internally and externally fertilising species alike. For instance, FRFs act as chemoattractants, guiding sperm towards the eggs in broadcast spawners and other external fertilisers, and are instrumental in maintaining species barriers by facilitating post-mating (or post-spawning) pre-zygotic isolation.

An outstanding question is whether or not any of these functions of FRF are retained in species with unconventional fertilisation strategies. One such example are bitterling – a group of small cyprinid fish distributed across Europe and Asia and reproductive parasites of freshwater mussels. Females deposit their eggs between the mussel gill lamellae by inserting a long ovipositor through the exhalant syphon inside the mussel gill cavity. Males ejaculate over the inhalant syphon and the water current

carries the sperm to the gills, where eggs are fertilised and embryos develop. Thus, while bitterling are external fertilisers, gametes are not released into the open water but contained within the mussel cavity and fertilisation is aided by mussel respiration, so it remains unknown whether bitterling FRF actively attracts sperm.

Using a recently developed multi-channel chamber, we test for the first time the role of bitterling FRF in sperm attraction, using the European bitterling *Rhodeus amarus* as a model species. We find that more sperm accumulates in the FRF channel compared to the control solution channel, suggesting that bitterling FRF retains chemoattractant properties despite the water current inside the mussel playing a key role in transporting sperm to the eggs. We also present preliminary results on interactions between *R. amarus* sperm and heterospecific FRF and discuss our findings in the context of reproductive parasitism evolution and hybridisation risk in this group of fish.



Stage-Specific Diets Shape Sex-Specific Plasticity and Evolution of Stress Resistance Across Age in *Drosophila melanogaster*

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MEETING ROOM 131, August 19, 2025, 2:00 PM - 5:00 PM

Background: Nutritional environments profoundly influence organismal physiology, particularly under environmental stress. Climate change and anthropogenic pressures are expected to increasingly disrupt nutrient availability and quality, potentially altering how organisms respond to abiotic stressors across life stages. While much is known about immediate physiological responses to diet, the interplay between diet-induced plasticity, ageing, and evolved stress resistance—especially in a sex-specific context—remains largely unresolved. We investigated whether stage-specific macronutrient environments drive age- and sex-dependent plasticity and long-term evolutionary shifts in multiple stress-resistance traits in *Drosophila melanogaster*.

Materials and Methods: We utilised a fully factorial larval × adult isocaloric diet design, varying in protein-to-carbohydrate (P:C) ratio, applied to a genetically diverse population of *D. melanogaster* over 70 generations. We assessed six stress-resistance traits—starvation, desiccation, heat shock, cold shock, and osmotic stress tolerance—at early, mid, and late ages in both sexes. Biochemical assays quantified energy stores (lipids, glycogen, and protein), and transcriptomic profiling (RNA-seq) was performed at all three age points to uncover diet- and age-dependent molecular signatures underlying phenotypic variation.

Results: Stress-resistance traits displayed strong age- and sex-specific responses to both larval and adult diets. No single diet combination maximised performance

across all traits or life stages, suggesting pervasive trade-offs. Notably, resistance to starvation and desiccation was enhanced by carbohydrate-rich larval diets in females, while protein-rich adult diets improved heat tolerance in males. Biochemical data revealed differential allocation of energy reserves across sexes and diets, consistent with observed performance outcomes. RNA-seq analyses uncovered age-dependent expression plasticity in stress-response and metabolic pathways, with significant enrichment of sex-biased transcripts and nutrient signaling regulators. Evolutionary responses to long-term diet regimes recapitulated some plastic responses but also revealed decoupling in certain traits, pointing to context-dependent adaptation.

Conclusions: Our results demonstrate that stage-specific diet composition has complex, sex-specific effects on stress resistance across age, mediated by both physiological reserves and gene expression plasticity. No dietary regime emerged as universally optimal, underscoring the role of environmental heterogeneity in maintaining life-history variation. This work highlights the ecological and evolutionary relevance of diet as a modulator of stress resilience and offers mechanistic insights into how nutritional environments may shape adaptive trajectories under environmental change. By integrating trait performance, molecular data, and evolutionary outcomes, our findings provide a comprehensive framework for understanding nutritional modulation of stress tolerance in natural and changing environments.



Symbioses between uncultivated microbes in the open ocean

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MEETING ROOM 117, August 18, 2025, 2:00 PM - 5:00 PM

Microbial symbiosis have a longstanding role in the evolution of ocean life. In modern times these interactions can be difficult to identify given the uncultivated nature of many ocean bacteria, archaea, and unicellular eukaryotes. We identify ecological specialization in the form of symbioses using culture independent methodologies at sea that allow us to recover and genome sequence physically interacting microbes. Our studies reveal a suite of symbioses that span a wide range of symbiont strategies. These strategies range from extreme genome reduction leading to obligate associations – to expanded genomes that encode pathways and proteins that facilitate interactions with multiple other living entities alongside the capacity to operate as a free-living cell. Some bring novel metabolic capabilities to hosts and modify possibilities for nutrient acquisition, however the conditions under which draw on host energetics balances with possible enhancements remain unknown. In several cases phylogenomic analyses demonstrate

that the symbionts of heterotrophic marine protists are the closest relatives identified (thus far) to symbionts of terrestrial insects. The marine lineages live in association with oceanic opisthokonts that are the closest living unicellular relatives of animals, and their related insect-symbiont lineages often act as pathogens when they encounter mammalian hosts. Moreover, our analyses show that among Rickettsiales and classical Legionellales association with Opisthokonta hosts appears to have evolved independently multiple times. Finally, some identified co-associations are detrimental to protistan hosts and likely contribute to their demise. Collectively, our studies point to widespread symbioses between the smallest unicellular eukaryotes in the oceans and divergent lineages of bacteria. These relationships underscore the important influences of symbioses on modern-day ecological and evolutionary trajectories of open ocean microbial communities.



The effects of costly telomere maintenance on lifespan - reproductive tradeoffs in sand lizards

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MEETING ROOM 131, August 19, 2025, 2:00 PM - 5:00 PM

Telomeres are DNA-protein structures that primarily protect chromosomes and serve multiple functions of gene regulation. When cells divide, telomeres shorten and their main repair system in ectotherms - telomerase - replaces lost nucleotide complexes ((T2AG3)_n in vertebrates). It remains a challenge to experimentally investigate resource requirements for telomere maintenance and its effects on lifespan-reproductive tradeoffs in the wild. In sand lizards (*Lacerta agilis*), we show that higher female investments into reproduction results in corresponding shortening of telomeres and that males have less frequent and less profound telomere shortening than females; a contributing factor to this may be

males' higher telomerase levels. To manipulate resource access for telomere maintenance, we exploit a pseudo-experimental opportunity to analyze 'onboard' resources long-term using lizards that drop their tails with fat and nutrient deposits when attacked by predators. Females with less resources due to regrown tails less often and less profoundly elongate telomeres. Adult lizards with the most TL elongation live the longest, females with the highest lifetime reproductive success shorten telomeres the most, whereas males with the most telomere elongation have the highest lifetime reproductive success. This suggests ongoing evolution of resource-constrained telomere maintenance.



The effects of sex-specific genetic variation and combined thermal and nutritional stress on body shape

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MEETING ROOM 131, August 19, 2025, 2:00 PM - 5:00 PM

Morphological diversity in animals largely arises from variations in the relative sizes of body parts, yet the contribution of genetic and environmental effects to variation in these allometric scaling relationships remain poorly understood. In this study, we explore how thermal, nutritional, and combined stressors influence the leg-body and wing-body scaling relationships in *Drosophila melanogaster*. Using 40 isogenic lines, we quantified individual-level (cryptic) scaling relationships under six distinct environmental conditions, enabling us to disentangle the contributions of genetic

variation and plasticity to allometric variation. We find significant sex-specific differences in intercepts but not slopes of wing-body and leg-body scaling relationships. Furthermore, shifts in the distribution patterns of cryptic scaling relationships under different environmental conditions suggest that the response to selection on body shape is dependent on the environmental context and is sex-specific. These findings provide novel insights into how complex environmental factors might shape morphological evolution.



The evolution of masturbation in birds

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A key assumption of research on sex is sexual behaviour is generally adaptive. However, one common sexual behaviour, masturbation, appears a Darwinian puzzle by potentially wasting valuable resources such as time, energy and, for males, sperm. We combined targeted surveys with information from published accounts to test hypotheses about why masturbation occurs, using a phylogenetically broad dataset on the presence or absence of masturbation across 120 bird species. We find masturbation is widespread in birds, but strongly phylogenetically conserved, typically being fixed (as present or absent) or nearly so within broad clades. Masturbation is no more common in juveniles than adults, suggesting

that it does not solely represent practice copulations before maturity and the widespread evidence in females suggests that maintaining fresh sperm in testes cannot be the single explanation. Species with indiscriminate matings are more likely to masturbate than socially monogamous species, or species with long-term pair bonds. Masturbation is more commonly reported in wild than captive birds, so does not appear to be a maladaptive response to captivity and rather part of a wider repertoire of sexual behaviours. Overall, our data are consistent with masturbation serving as a sexual outlet, a means of increasing reproductive success, or both.



The evolutionary history of a commensal species: lessons from adapting to the Anthropocene

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Human activity has altered the evolutionary trajectories and ecological circumstances of nearly every species on Earth. Commensal species are an extreme example to adaptation to anthropogenic niches, benefitting from a close association with human activity. What can the evolution of a commensal life history tell us about how species adapt to environmental change driven by humans?

House sparrows (*Passer domesticus*) are a hugely successful human-commensal species, occurring on nearly every continent, largely due to human introductions. Despite its close association human society, surprisingly little is known about the evolutionary history of this species, how it has evolved to fit a tight anthropogenic niche and the evolutionary consequences of its rapid global spread. Our work has focused on addressing this.

With resequencing data from over 1200 *Passer* sparrows, we first reconstructed the evolutionary history of the house sparrow. Our results suggest the species likely adapted to a human niche, potentially more than once, in Central Asia during the last 10,000 years. We used phenotypic data including micro-CT 3D scanning to compare skull morphology between human commensal and wild sparrow populations. We identified clear divergence in skull morphology, beak shape and an increased brain size in the commensal house sparrow populations. A major signature selective sweep in the house sparrow genome encompasses two major candidate genes; COL11A1 – which regulates craniofacial and skull development and AMY2A which is

linked to adaptation to high-starch diets in humans and dogs. Functional analysis of COL11A1 confirms its role in skull development and we show evidence of copy number variation at AMY2A.

Our next aim was to investigate the more recent global spread of the species. Native to Eurasia, the house sparrow was introduced to the Americas, Australasia and Sub-Saharan Africa in the 19th and 20th centuries. Using resequenced genome data from across this range, we have reconstructed the population genomic signatures of these introductions. Our findings point to introductions from two divergent lineages of house sparrow to different parts of the world as well as the rapid evolution of genetic differentiation among introduced populations. We further investigated adaptation to climatic variation across the introduced ranges of the species in North America and Australia. We identify several candidate genes linked to growth, thermoregulation and thermal tolerance, suggesting introduced populations have adapted to new environmental conditions in less than 150 generations.

Our work identifies phenotypes and genes involved in rapid adaptation in parallel to anthropogenic change across the native and introduced range of the house sparrow. With a global distribution spanning a wide range of climatic conditions and a close association with human society, we suggest the house sparrow is an ideal model species for understanding adaptation in the Anthropocene.



The genetic basis of multiple independent origins of a placenta

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MEETING ROOM 132, August 18, 2025, 10:30 AM - 12:30 PM

How do new cells, new tissues, and entire new organs arise during evolution? Our lab investigates these questions using an organ that has evolved independently many times and is remarkably diverse: the placenta. The placenta forms through the fusion of embryonic and maternal tissues, enabling the transfer of nutrients, gases, waste, and more during gestation. In placental species, such as mammals, the mother gestates her offspring inside her body and nourishes them via a placenta. Remarkably, placentas have evolved more than 100 times independently among vertebrates, including once in mammals. Within a family of small live-bearing fishes known as Poeciliidae, at least nine independent origins of the placenta have occurred. Poeciliid placentas evolved relatively recently in evolutionary time, with some originating less than 10 million years ago. This makes them excellent models for studying how new organs emerge during evolution. We have combined whole-genome sequencing, single-cell RNA

sequencing, spatial transcriptomics, and imaging to uncover the genetic and developmental basis of four independent origins of a placenta in Poeciliids. Our research revealed that the evolution of a novel cell type unique to Poeciliids allowed this family to transition from egg-laying to live-bearing through egg retention. This new cell type was subsequently co-opted to make up the bulk of the placenta in the four placental species and functionally diversified to deliver key placenta functions such as nutrient transport and hormone response. The four independently evolved placentas are morphologically distinct, varying in cell composition and organisation. In two of these origins, we identified the emergence of additional novel cell types. Our work illustrates the diverse genetic and developmental paths underlying the evolution of new organs, even among closely related species, and supports a link between cell-type innovation and the origin of new organs.



The impact of population substructure on SMC-based inference tools analyzed through extensive demographic simulations on spatially complex demographic models

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The increasing availability of whole genome sequences has significantly advanced our ability to reconstruct demographic histories and evolutionary processes with greater resolution. These data have facilitated the application of powerful inference methods, many of which rely on Sequentially Markovian Coalescent (SMC) models to infer past population dynamics. However, while these models have been widely used to estimate changes in effective population size over time, their accuracy and reliability can be compromised when applied to populations with complex structures. In particular, structured populations introduce deviations from the assumptions of panmixia, potentially leading to biased inferences and misinterpretations of demographic history.

In this study, we systematically assess the impact of population substructure on SMC-based inference methods and explore how these biases arise under different demographic scenarios. To achieve this, we perform extensive coalescent simulations

designed to capture the effects of structured demographic histories, enabling a detailed evaluation of the biases introduced by population stratification.

Our results demonstrate that the magnitude and direction of these biases depend on factors such as the sampling scheme and key parameters governing the underlying population structure. Furthermore, we show that demographic interpretations based on SMC-inferred coalescent rates are inherently constrained by the model's assumptions, which often overlook deviations from panmixia. These findings emphasize the need for caution when applying SMC-based methods to structured populations and highlight the importance of developing refined approaches for more accurate demographic inference.

Furthermore, we explore the informativeness of novel summary statistics from whole genome sequences and propose a framework that couples Deep Learning with the SMC principles to predict the level of structure and the variation of migration in structured models.



The origin and spread of sorghum in Africa: a multidisciplinary perspective integrating genomics, archaeobotanical and ethnolinguistic data

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MEETING ROOM 118+119, August 19, 2025, 2:00 PM - 5:00 PM

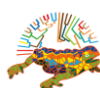
Understanding the origins of major crops and their early diffusion is crucial to reconstructing the history of human societies and their interactions with the environments. In this study, we focused on sorghum, a crop of major worldwide economic and cultural importance. Archaeobotanical evidence suggests that its domestication originated in eastern Sudan during the fourth millennium BCE but its early diffusion remains unclear. We used a genomic approach, combined with archaeobotanical and ethnolinguistic data, to refine the spatial and temporal origin of cultivated sorghum, and to better understand its spread across Africa.

We built a probability map of sorghum domestication using genomic data combined with spatial Bayesian modeling. Our findings pinpoint its centre of domestication to a region encompassing Eastern Sudan and Western Ethiopia, which aligns closely with recent archaeobotanical evidence. Our genomic-based model, calibrated on archaeological remains, suggests that the beginning of the expansion of sorghum agriculture took place around

4,600 years ago. Our results suggest that its expansion occurred simultaneously westward and southward from the centre of domestication, at similar speed.

Importantly, the structure of sorghum populations mirrors ethnolinguistic groups at different scales, suggesting a co-diffusion of human social groups and sorghum populations. Their association remains even at fine linguistic classification, suggesting that sorghum populations were maintained by complementary processes, such as local adaptation or social barriers to seed exchange.

Our study provides an archaeology-independent, genomically-based estimation of sorghum's domestication centre and highlights the intertwined dynamics of biological and social factors in shaping crop diversity. Thus, by integrating interdisciplinary data, the study highlights the advantages of combining biological and social science perspectives to understand crops - and, more broadly, domesticated organisms - as biological, social and historical entities.



The role of song in population divergence: Insights from the La Palma's Canary Islands Chaffinch

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Understanding the speciation process is one of the most pressing questions in evolutionary biology. In songbirds, vocalisations are important in individual and species recognition and are crucial in sexual and social communication. Populations that diverge in song may fail to recognise each other, leading to assortative mating and, potentially, speciation. Therefore, song may constitute a premating barrier to reproduction. The chaffinches from La Palma (Canary Islands, Spain) constitute a clear case of incipient lineage splitting with populations that inhabit pine forests being genetically and morphologically differentiated from cloud forest populations. However, song characteristics of these populations have not been studied, so the role of song in their divergence into different lineages that have remained distinct in spite of being in close proximity from one another remains unclear. We aimed to characterise and quantitatively compare acoustic traits between populations from the two habitats, focusing on song composition, syllable diversity, and shared syllables, and we also tested experimentally whether their song differences could act as a behavioural isolating mechanism. By recording songs of both populations across their distribution

ranges and using a novel approach to directly capture the frequency modulation of syllables, we found differences in the songs of individuals that were associated with forest type, with results revealing three well-differentiated groups: pine, cloud, and ecotone between these two. Our playback experiments used songs from the same and different habitats, as well as songs from a close relative, the Common Chaffinch from mainland Europe, and the reaction of the territorial individuals was registered. Both probability and intensity of response to songs of the close relative species and to the different-habitat birds were weaker than to songs of the same-habitat birds, but differences observed in the second case were not statistically significant. Although song differences seem not to be strong enough to elicit marked reactions between territorial males from opposite habitat types, further experiments will be necessary to test female response to these songs in order to determine if they convey lineage-specific information or not. This study adds then to the debate over the role of song differentiation in driving the evolution of reproductive isolation, and whether song acts as a barrier only when other phenotypic differences are already well established.



Timescale and genetic linkage explain the variable impact of defense systems on horizontal gene transfer

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Prokaryotes have evolved a wide repertoire of defense systems to prevent invasion by mobile genetic elements (MGEs). However, because MGEs are vehicles for the exchange of beneficial accessory genes, defense systems could consequently impede rapid adaptation in microbial populations. To shed light on the complex interplay between MGEs, defense systems, and horizontal gene transfer (HGT), we studied how defense systems impact gene exchange rates and MGE abundances in the short term and long term.

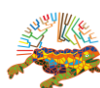
By combining comparative genomics and phylogeny-aware statistical methods, we quantified the association between the presence of seven widespread defense systems and the abundance of MGEs in the genomes of 196 bacterial and one archaeal species. We also calculated the differences in the rates of gene gain and loss between lineages that possess and lack each defense system.

Our results show that the impact of defense systems on HGT is highly taxon and system dependent, with CRISPR-Cas standing out as

the defense system that most often associates with a decrease in HGT. Timescale analysis reveals that cross-species variability is partly explained by differences in genetic linkage between defense systems and MGE. On one hand, defense systems must persist in a lineage for a relatively long time in order exert an appreciable negative impact on HGT. On the other hand, at short evolutionary times, defense systems, MGE, and gene gain rates tend to be positively correlated due to recent co-transfer events [1].

Given the high turnover rates experienced by defense systems, we propose that the inhibitory effect of most defense systems on HGT is masked by their strong linkage with MGEs. These findings help explain the contradictory conclusions of previous research by pointing at mobility and within-host retention times as key factors that determine the impact of defense systems on genome plasticity.

[1] Liu, Botelho, Iranzo (2025) *Genome Res.*, 35(2):268-278



Towards the end of the lipid divide?

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MEETING ROOM 117, August 21, 2025, 2:00 PM - 5:00 PM

The lipid divide distinguishes archaea from bacteria and eukaryotes on the basis of membrane features. Speculated instability of mixed membranes have led to their expected instability, so-called the 'lipid divide'. However, bacteria and archaea have the potential to synthesize lipids of the other type, resulting in the formation of mixed membranes. Mixed membranes have also been shown to be stable and viable when

induced in cells. Detection of a third type of lipids with archaeal characters in bacteria suggest intermediary lipids exist. In addition, Asgard archaea, the closest prokaryotes to eukaryotes, appear to lack G1P synthesis capacity but to be able to synthesize G3P instead. Altogether, this suggests weakening of the lipid divide with possible transitions, and even its non-existence. Here I review the arguments weakening the lipid divide and the implications of its possible bridging.



Trade-offs beget trade-offs: Causal analysis of mammalian population dynamics

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Survival and reproduction strategies in mammals are determined by trade-offs between life history traits. In turn, the unique configuration of traits that characterizes mammalian species gives rise to species-specific population dynamics. The dependence of population dynamics on life history has been primarily studied as the relationship between population density and size-related traits. With the recent accumulation of genomic data, the effective population size (number of breeding individuals; N_e) over the last 100-800 kya has become quantifiable for a large proportion of mammals. Using phylogenetic path analysis, we compared the dependence of population density and N_e on eleven traits that characterize mammalian allometry, diet and reproduction. We found variable trait impacts on these two metrics of population dynamics across different phylogenetic, ecological and conservation classifications

of mammalian species. Brain size had a negative effect on density but a positive effect on N_e , especially in primates and carnivores. Diet specialization had a negative effect on both density and N_e . The relationship between N_e (but not density) and several traits showed biome-related gradients. We found that critically endangered species have life history strategies that impede population recovery, as reflected by the strong positive dependence of both density and N_e on generation length. Our findings thus demonstrate that trade-offs characterizing life history evolution are not only trait-specific, but are also present between different metrics of population dynamics. This challenges the static nature of the “energetic equivalence” rule and has major implications for selecting the appropriate population metric in species conservation and restoration strategies



Uncovering Introgression Events in Potato Domestication via TE Landscape Analysis

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Background: The common potato (*Solanum tuberosum* L.), a tetraploid crop, originated from wild species native to South America. Domesticated around 10,000 years ago in the Andes, potatoes were introduced to Europe in the 16th century and spread globally thereafter. This complex evolutionary history, shaped by hybridization during and after domestication, involves recurrent introgression from wild species into cultivated varieties. However, the specifics and distribution of these events remain unclear.

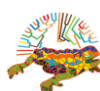
One genomic consequence of introgression is the incorporation of transposable elements (TEs) from divergent wild genomes. These TEs can provide signatures of historical introgression events. In this study, we aim to identify introgressed regions in the cultivated potato genome and determine their wild donor species. More specifically, we aim to infer the timeline of introgression events based on the divergence of TE copies, with older copies residing in introgressed blocks and newer copies integrated elsewhere in the genome.

Materials and methods: We analyzed a comprehensive dataset of 217 samples that represent 71 wild potato species from four major phylogenetic clades, alongside multiple haplotype-phased assemblies of cultivated tetraploid potatoes. To identify introgressed regions, we applied a window-based approach using SNP distance matrices to compare cultivated and wild potato genomes. We annotated Long Terminal Repeat (LTR) retrotransposons and analyzed their presence, distribution, and enrichment within introgressed regions

across the genome. By comparing TE abundance in introgressed regions and assessing genetic diversity within TE families, we traced the contribution of wild species to the cultivated genome and estimated the relative timing of each introgression event.

Results: Our analysis revealed widespread introgression from wild potato species into the cultivated potato genome. Wild species more closely related to cultivated potatoes were more frequently associated with introgressed regions. However, species from more distantly related clades also made substantial contributions in several genomic regions, suggesting recent introgression. In addition, the dominant contributing species varied across chromosomes and even across haplotypes, indicating the importance of using haplotype-resolved assemblies in detecting introgressed regions. Lastly, TE analysis revealed enrichment of specific LTR retrotransposon families within introgressed regions, with species and chromosome-specific enrichment patterns.

Conclusions: This study provides a detailed view of introgression from wild potato species into the cultivated potato genome. Furthermore, by comparing the divergence between LTR families in the introgressed regions, we were able to estimate the relative timing of these introgression events. This approach offers valuable insights into the genomic consequences of introgression during domestication, particularly in relation to the population dynamics of TEs and their role in shaping the adaptive potential of cultivated potatoes.



Uncovering the genetic and mechanistic basis of phenotypic polymorphisms in island populations of wall lizards

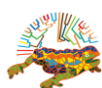
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Island populations offer a unique lens into the mechanics of evolution. In these isolated environments, the effects of genetic drift and selection are often magnified. However, there is lack of research integrating both genomic, phenotypic and ecological data, limiting our ability to uncover the mechanistic basis of complex phenotypic traits. Here, we addressed this gap by assessing the drivers of animal shape, colour and symmetry in island populations of wall lizards (*Podarcis siculus*). We performed a genotype-phenotype association study to identify the genome-wide variants determining head shape (shape configurations of cephalic scales), dorsal colour (colouration spectra of dorsal skin), and the occurrence of asymmetric phenotypes in epidermal structures (ventral scales, femoral pores). Then, we evaluated the respective influence of multiple predictors on phenotypic variation. Sexes differed in head shape and skin colour, and

these differences were consistent across environments. Genotypic effects were strong, both for neutral genetic structure and adaptive genetic variance (G×P interaction), which was conditioned by the environment (environment-dependent G×P interaction). Concerning animal symmetry, we found different responses between sexes, with reduced male asymmetry in stressful environments (predation pressure) and for higher values of individual body condition, suggesting that asymmetry decreases individual performance. Male asymmetry was further associated with higher levels of relatedness between individuals, suggesting inbreeding depression. Our study uncovers that phenotypes are shaped by a dynamic interplay of forces, from population history to local adaptation, plastic responses and developmental instability. This highlights how complex it is to predict the evolutionary trajectories of populations, especially given their past and current genetic contexts.



Under pressure: Adaptation of opsin proteins shaped by interacting selective factors

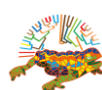
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Identifying the molecular mechanisms underlying adaptive phenotypic changes is essential for understanding species divergence. The visual system is an ideal model for studying these adaptations due to the well-documented genetic basis of its characters and their adaptive consequences. While many environmental factors shape visual systems, most studies have concentrated solely on the role of light. This leaves critical gaps in understanding how multiple selective forces interact and shape visual systems. In aquatic systems, depth is a strong predictor of visual evolution because light availability decreases rapidly with increased depth. However, depth is also correlated with other factors, such as hydrostatic pressure, that also strongly affect vision but are often overlooked. To evaluate how multiple selective forces shape visual systems, we use 107 genomes from a

diverse genus of marine fishes (Rockfish; Sebastes). We analyze sequence and expression-level variation in opsins — the proteins responsible for detecting specific wavelengths of light. We use a phylogenetic comparative approach to (a) test for changes in amino acid sequences and gene expression that tune visual systems to the available light spectrum and (b) examine amino acid sequences that enhance protein stability and reduce protein compressibility under high hydrostatic pressure. We present preliminary findings on how the rod opsin (RH1) and one cone opsin (LWS) respond to these selective forces through changes in amino acid sequence. Understanding how visual systems in rockfish simultaneously respond to different environmental factors will help identify general patterns of opsin, visual system, and species diversification across aquatic taxa.



Understanding the determinants of divergence and reproductive isolation in marine fishes using a comparative speciation genomics approach

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Background: The rate at which new species form varies widely across the tree of life, raising a fundamental question: why do some lineages split, diverge and develop reproductive isolation faster than others? Speciation is driven by diverse population-level mechanisms shaped by ecological and life-history factors. However, their ultimate effects on speciation remain poorly understood.

Materials and methods: Here, we examined patterns of divergence and reproductive isolation in 20 marine fish species, each split into an Atlantic and a Mediterranean lineage. These species share a common biogeographic history and similar genome architecture but differ in their life histories. We sampled and sequenced the whole genomes of 20 individuals per species, collected at two locations per basin, both within and outside the suture zone between the Atlantic Ocean and the Mediterranean Sea. We measured and compared several important genetic components of speciation: the reservoir of ancestral genetic diversity before population split, the time of split, the accumulation of genetic differentiation over time, the amount of gene flow, and the resulting degree of semipermeability — a genome-wide genetic signature of reproductive isolation.

Results: First, we described a gradient of divergence among the 20 species pairs, ranging from near panmixia to almost complete arrest of gene flow between the two basins. This gradient was reflected in genome-wide patterns, from no

differentiation at any locus to heterogeneous variation and, ultimately, to strong genome-wide differentiation. Yet, introgression metrics and ABC demographic modelling revealed signs of gene flow across the Atlantic-Mediterranean suture zone along the entire gradient. By inferring the Ancestral Recombination Graph (ARG) for each species, which summarizes the full history of recombination and coalescence across the genome, we found distinct divergence histories. Using simulations, we show that the ARG can be used to estimate the level of reproductive isolation based on the proportion of regions exhibiting lineage sorting despite gene flow.

Genetic components of divergence and reproductive isolation were found to correlate with life-history strategies summarized by gradients of growth and parental investment. Notably, the classical slow-fast continuum of fish life-histories was identified as the main driver of divergence. Slow-living species with generally longer pelagic larval durations (used as a proxy for dispersal capacity), tended to show lower divergence, higher gene flow, and ultimately lower genetic differentiation and lower reproductive isolation.

Conclusions: Overall, our comparative analysis of 20 species from a shared Atlantic-Mediterranean biogeographic context shows that life-history strategies are key modulators of the genetic drivers of speciation, shaping ancestral variation, lineage sorting, gene flow, and eventually, reproductive isolation.



Unicellular regulatory landscapes and the origins of animal multicellularity

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The origin of animals is one of the most persistent questions in evolutionary biology, particularly regarding how unicellular ancestors transformed into complex multicellular organisms. This change required not only new genes but also changes in their regulation. Recent studies in early branching animals have revealed the presence of distal enhancer-like elements, key regulatory features that enable complex spatial and temporal control of gene expression. However, these elements have not been identified in the unicellular holozoans analysed so far, *Capsaspora owczarzaki* (Filasterean) and *Salpingoeca rosetta* (Choanoflagellate), leaving a critical gap in our understanding of how complex regulation evolved.

Our research addresses this gap by studying the diversity of regulatory frameworks in a broader range of unicellular holozoans, particularly the Ichthyosporeans, Corallochytreans, and Filasterean, lineages closely related to animals. These taxa are crucial for reconstructing the ancestral genomic and regulatory context from which animals emerged.

We combine chromatin accessibility (ATAC-seq) data with CUT&Tag profiles of histone modifications to map regulatory elements in multiple species. Furthermore, we have generated new high-quality genome assemblies obtained through long reads, which serve as reliable references for identifying and comparing regulatory features across taxa.

Our findings reveal significant variability in the presence, number, and types of putative regulatory elements among unicellular holozoans. In particular, the ichthyosporean *Abeoforma whisleri* shows a high diversity of accessible chromatin regions and histone marks associated with gene regulation,

suggesting a more complex regulatory toolkit than previously appreciated in unicellular lineages. In contrast, *Creolimax fragrantissima*, also an ichthyosporean, exhibits a more optimised regulatory architecture, with fewer and less diverse regulatory signatures.

In addition to chromatin landscapes, we are investigating the presence and activity of transposable elements (TEs) in these unicellular genomes. TEs are increasingly recognised not only as sources of genomic innovation but also as contributors to the evolution of regulatory elements. In animals, many enhancers and transcription factor binding sites originate from ancient TE insertions. By analysing the chromatin composition and context of TEs in unicellular holozoans, we aim to assess their potential role in shaping regulatory complexity before the emergence of animals. Preliminary analyses reveal species-specific differences in TE content, with some lineages showing evidence of recent activity and possible incorporation into regulatory networks.

These results suggest that there is no single, conserved regulatory landscape among unicellular holozoans. Instead, these organisms exhibit a mosaic of regulatory strategies that may reflect different ecological roles, life-history complexities, or evolutionary histories. Importantly, our findings imply that elements of complex gene regulation—and perhaps their association with mobile genetic elements—predate the emergence of animals and may have assembled gradually and unevenly across lineages. By expanding the taxonomic and functional scope of the regulatory landscape analysis, this work brings us closer to understanding how the genomic foundations of animal multicellularity were built, step by step.



Unusual sex chromosome structure of *B. pusilla* and the evolution of sex chromosomes in liverworts

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MEETING ROOM 115, August 21, 2025, 2:00 PM - 5:00 PM

Sex chromosomes often evolve from autosomes through the emergence of a non-recombining sex-determining region which is followed by the expansion of recombination suppression leading to chromosomal differentiation. As a result, older sex chromosomes often exhibit significant size asymmetry (heteromorphy) and are either significantly larger or smaller than their autosomal counterparts. In organisms that express sex during the haploid phase of their life cycle, such as liverworts, sex is determined by haploid U (female) and V (male) chromosomes, which are often heteromorphic and smaller than autosomes. However, several liverwort species that evolved dioecy a long time ago possess homomorphic sex chromosomes, the evolutionary origins of which remain largely unresolved. In this study we investigated the evolutionary origin of sex chromosomes in *Blasia pusilla* by generating genome assemblies for the first liverwort species possessing homomorphic U/V sex chromosomes. Having assembled and annotated the genomes of male and female individuals, we found that the nine chromosomes were of a similar size, unlike in other liverworts which possess eight large autosomes and one small sex chromosome. The sex chromosome of *B. pusilla* showed a

structure unusual in liverworts, consisting of a recombining pseudoautosomal region and a short, non-recombining region located terminally that determines sex. Furthermore, we found that the suppression of recombination in the sex-determining region predates the divergence of *M. polymorpha* and *B. pusilla*, indicating that it was already present in their common ancestor, the common ancestor of most liverworts. This genomic configuration suggests two potential evolutionary pathways:

(i) The homomorphic sex chromosomes of *B. pusilla* could be ancestral to liverworts. In this case, the highly heteromorphic and remarkably small sex chromosomes typical of most liverworts could have evolved through chromosomal fission of the sex-determining region. (ii) Alternatively, small, highly heteromorphic sex chromosomes may be ancestral to liverworts, in which case homomorphic sex chromosomes might have originated via fusion with an autosome. Phylogenetic distribution of species with homomorphic sex chromosomes favor the first hypothesis suggesting that homomorphic sex chromosomes are ancestral to liverworts. Using this information, we present a mechanistic model of the evolution of sex chromosomes and sex determination in liverworts.



Unveiling the Evolutionary Dynamics of the X Chromosome in Aphids

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MEETING ROOM 115, August 21, 2025, 2:00 PM - 5:00 PM

Sex chromosomes often exhibit distinct evolutionary dynamics compared to the rest of the genome. In many organisms, the X chromosome is thought to evolve faster than autosomes (“faster-X” effect) due to the exposure of recessive alleles in hemizygous males and differences in effective population size. Aphids (family Aphididae) present an XX/X0 sex chromosome system and undergo cyclical parthenogenesis, making them a compelling model to examine X-linked evolution. Previous studies on a few aphid species hinted that X-linked genes may evolve more rapidly and carry more male-biased genes than autosomes. However, it remains unclear whether these patterns hold across a broad phylogenetic scale and how they relate to the origin of novel genes. We hypothesize that the X chromosome experiences accelerated sequence divergence across the Aphididae, promoting the emergence of novel genes.

We conducted a comparative genomic analysis encompassing 47 aphid species spanning the Aphididae family. Chromosome-level genome assemblies were analyzed to identify orthologous gene relationships and to assess macro-synteny conservation between X chromosomes and autosomes. We estimated sequence divergence using multiple whole-genome alignment strategies, comparing overall sequence identity between X chromosomes and autosomes across species. Novel genes were detected based on absence of orthology with other aphid species. We also integrated male and female transcriptome (RNA-seq) data from multiple representative species to determine sex-biased expression patterns and further analyzed their evolutionary rates. We also explored the potential contribution of transposable element (TE) activity to novel gene emergence.

Our analysis shows that the X chromosome maintains conserved macro-synteny across aphids despite many internal rearrangements, while autosomes experience both within and between chromosome changes. Despite this conserved macro-synteny, X-linked sequences exhibit significantly elevated divergence between species compared to autosomal regions, consistent with a genome-wide “faster-X” effect. We identified hundreds of novel genes in each representative species, with most of them located on the X chromosome. Preliminary investigations suggest that transposable elements may contribute to the origination of some novel genes. Furthermore, by integrating sex-specific transcriptomes, we validated the faster evolutionary rate of X-linked genes, expanding previous observations from the pea aphid to a broader range of species across the Aphididae family. Ongoing work is investigating the expression patterns of novel genes on the X chromosome.

Our findings provide strong evidence that the X chromosome in aphids evolves faster than the rest of the genome and fuels genetic innovation. This study is the first to demonstrate at a family-wide scale that the “faster-X” phenomenon extends to overall sequence divergence across many species. The enrichment of novel, male-biased genes on the X chromosome, possibly influenced by TE activity, highlights the unique role of genome architecture—in particular, sex chromosome structure and inheritance—in shaping evolutionary trajectories and generating new genetic diversity in Aphididae. This work was supported by the ALTEREVO ERC project (Grant No. 101054340).



Using a Meta-Omics Approach to Explore Opsin Diversity in Zooplankton

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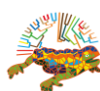
MEETING ROOM 132, August 18, 2025, 10:30 AM - 12:30 PM

We investigated the diversity and evolution of opsins using meta-omic data from the Tara Oceans and Tara Polar Circle expeditions, comprising over 272 sampling locations and reaching depths of up to 1000 meters, representing one of the most comprehensive metagenomic and metatranscriptomic resources currently available. Employing a suite of bioinformatics tools, including BLASTp, MCL clustering, gene annotation, and maximum likelihood phylogenetic analyses, we mined the Tara Oceans Eukaryotic Genomes (SMAGs) and the Eukaryote Gene Catalog (MATOU) datasets to characterize opsins across diverse metazoan lineages and opsin subfamilies. Our analysis revealed that most opsin sequences belonged to arthropod and vertebrate visual opsins.

Notably, we observed a significant representation of medium-wavelength (MW) opsins in arthropods, specifically MW1 in malacostracans and branchiopods, and MW2 in copepods. Among vertebrates, the

majority of sequences corresponded to the green-sensitive opsin Rh2. Despite that, we identified sequences from all major opsin subfamilies, including r-opsin, c-opsin, xenopsin, and Group-4 opsins, across key zooplankton taxa, including groups that have historically been understudied in this context, such as chaetognaths, rotiferans, tunicates, and acoelomates. Despite the broad taxonomic coverage, no novel opsin families were discovered. Our phylogenetic analyses corroborate existing opsin phylogenies, placing r-opsins as basal, with xenopsins clustering with c-opsins, and Group-4 opsins forming a sister clade to the xenopsin/c-opsin group.

Overall, our work provides a robust taxonomic annotation of opsin sequences derived from the Tara Oceans datasets, offering valuable resources for future studies on the molecular evolution and ecological distribution of light-sensing proteins in marine zooplankton.



Why do the songbirds perform tap dance?: Behavioral mechanisms and functions of mutual multimodal courtship displays

Ota N^{1,2}, Soma M³, Gahr M²

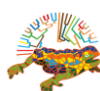
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MEETING ROOM 118+119, August 19, 2025, 2:00 PM - 5:00 PM

Birds exhibit diverse and elaborate courtship displays that include multiple behavioral components. Estrildid finches are one of the most studied songbird families for acoustic communication. They often combine their vocalizations (i.e., songs) with body movements (i.e., dance) during courtship. Past literature has provided detailed descriptions of dance components, but compared to songs, there are still few empirical studies on the behavioral mechanisms and functions of dancing. Our study species, blue-capped cordon-bleus (*Uraeginthus cyanocephalus*), are socially monogamous Estrildid finches and perform characteristic dance displays in both sexes. During courtship, they hold a piece of nesting material, then repeatedly bob up and down and sing songs several times. We have previously found that they perform human tap-dance-like high-speed movements, tapping their legs on the perch two to six times in a single bobbing. Their behavior is

interesting not only because both sexes perform almost the same display but also because their dance movements can produce sounds and vibrations. Here we present our findings on the dance behavior of cordon-bleus in both captive and wild conditions. We revealed that blue-capped cordon-bleus adjust their tap-dance-like behavior according to the presence and distance of surrounding individuals. Specifically, they danced frequently when potential mates stayed on the same perch where vibrations could be transmitted, and the surrounding individuals observed the courting interaction. Our studies suggest that the sounds and vibrations of dancing can function as communication signals and have multifaceted functions depending on the social and environmental contexts. It underscores the importance of studying the non-vocal aspects of songbird courtship displays towards a better understanding of animal communication and its complexities.



A curated great ape genome diversity panel and a selection screen

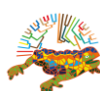
Han S¹, Huang X¹, Kuhlwiilm M¹

¹University Of Vienna

POSTER SESSION 3 - **P03.243**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Studying the genetic diversity of great apes is a crucial part for understanding their evolution and relevant for comprehending human evolution. Whole/genome diversity data on the three great ape clades (Pan, Gorilla, Pongo) has been published across multiple studies over more than one decade. However, unlike in humans, no comprehensive dataset on this diversity has been available. We have curated a dataset of 203 high coverage (>12-fold) whole genomes, including 77 chimpanzees, 14 bonobos, 77 gorillas and 35 orangutans. We thoroughly assessed the quality of this

dataset, providing an important resource for evolutionary studies. We leveraged this dataset for refining the genomic landscape of natural selection in these species. We applied a screen for signatures of positive selection as well as long-term balancing selection across species. We also quantified the distribution of fitness effects and assessed the correlations of natural selection across species using allele frequency spectra and joint allele frequency spectra. These analyses offer a better understanding of their evolutionary history.



A formal theory of group-level adaptation for obligate eusociality

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POSTER SESSION 1 - **P01.068**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Darwin argued that natural selection leads organisms to appear as if they are striving to maximise their fitness. This idea is readily recognised at the individual level, but such adaptive design may also manifest at other levels of biological organisation. Previous work has formalised the idea that social groups can be viewed as adaptive individuals in their own right—i.e., ‘superorganisms’—under the assumption that within-group selection is absent and that there is no class structure. However, the original and most common biological use of the term ‘superorganism’ is in reference to eusocial insect colonies in which members exhibit striking class structure in the form of reproductive division of labour. Accordingly,

although obligately eusocial colonies are regularly conceptualised as having the capacity for colony-level adaptation, current formalisms are unable to fully support this idea. Here, we develop a formal theory of group-level adaptation for obligately eusocial colonies, by establishing mathematical correspondences that connect the dynamics of natural selection—as described by Price’s equation—to the mathematics of optimisation—wherein the colony is considered a fitness-maximising agent—under a range of assumptions as to which members of the colony control its phenotype and the degree to which they are genetically related.



A new model of background selection improves our understanding of human genetic diversity

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POSTER SESSION 1 - with Aperitif, **P01.147**, August 18, 2025, 5:00 PM - 7:00 PM

The effect of negative selection on linked genetic diversity has received much attention over the past three decades. Classic background selection theory concerns sufficiently strong selection that the frequencies of deleterious variants stay close to mutation selection balance. However, the model breaks down as selection weakens and drift allows them to segregate more freely. Recent applications were able to explain a large portion of the variation in diversity across the human genome, but their inferences of selection parameters are incompatible with the literature. This conflict likely stems from two key limitations. First, current background selection models are inaccurate in the most critical region of parameter space (for scaled selection coefficients Ns around -1), where the reduction in diversity is sharpest; second, they assume a constant population size over time. Here, we develop a new method based on the Hill-Robertson system of two-locus statistics that we have recently generalized. Our model regards the evolution of a neutral locus linked to a constrained locus at arbitrary recombination distance and population history. Our goal is to quantify the relative reduction in heterozygosity due to linked selection (B-value). We treat the joint effect of multiple selected loci independently, but we show that interference among them is well captured through local rescaling of mutation, recombination and selection in an iterative procedure that converges quickly. We further accommodate Nordborg's (1997) model to population size changes, thus bridging the gap between weak and strong

selection. We show via simulations that our formulation leads to accurate B-value predictions, including the critical region of $Ns \sim -1$. We explore the temporal dynamics of linked selection under non-equilibrium demography and demonstrate that patterns of diversity can be misinterpreted by other models. Specifically, neglecting population history may bias prediction of B-value maps. These biases then carry over to downstream inferences of the distribution of fitness effects and deleterious mutation rates. Therefore, jointly modeling demography and linked selection improves our understanding of the genome-wide landscape of diversity. We finally use our new model to study human populations. Its increased accuracy, coupled with a state-of-the-art mutation map, allowed us to predict B-values using selection coefficients inferred from the site frequency spectrum, avoiding the circularity inherent in other efforts. Even without model fitting, our predictions describe genetic diversity comparably to previous studies. We also uncover a previously uncharacterized heterogeneity across the genome: the variance in heterozygosity accounted for by linked selection varies from $\sim 40\%$ to $\sim 80\%$ among chromosomes. Adding mutation rate variation as an explanatory variable raises this figure beyond previous estimates. Causal inference allows us to tease apart the direct and indirect effects of mutation and linked selection in human genomes, and quantify their relative importance. We anticipate that our new model will help refine the picture of linked selection in humans and other species.



A temporal analysis of sockeye-kokanee hybridization, behaviour, and fitness following a re-introduction program

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POSTER SESSION 3 - **P03.260**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Stocking programs have been widely implemented to restore extirpated fish species to their historical ranges, but must include careful consideration of potential hybridization dynamics. The sockeye salmon (*Oncorhynchus nerka*) reintroduction project in Skaha Lake (British Columbia, Canada) provides an excellent opportunity to investigate the outcomes of hybridization between anadromous (migratory) sockeye and resident kokanee over a long-term time series to inform larger-scale restocking and habitat restoration initiatives. Prior research established the presence of hybrids, with most hybrid spawners exhibiting a resident life-history. However, it remained unclear whether this skew towards residency resulted from: 1) hybrid smolts that exhibit an anadromous life history having reduced fitness compared to sockeye; and/or 2) hybrid smolts not migrating and predominantly exhibiting a resident life history.

Materials and methods: Here, we paired a cohort-based sampling design (age-0 fry, age-1 smolts, and adult spawners) with SNP genotyping, otolith microchemistry analysis, and morphometrics to: 1) reconstruct stock proportions of sockeye, kokanee and hybrids (F1, Backcross-sockeye, Backcross-kokanee) by extending the long-term time series within the Skaha Lake system, and 2) investigate the fitness and behavioural outcomes of sockeye-kokanee hybridization. DNA was extracted from tissue samples (fin or operculum punch) and subsequently genotyped using a 342 SNP GT-seq panel previously developed to assign individuals to advanced hybrid classes, with assignments

made in NEWHYBRIDS. Paired with these genetic assignments, a subset of spawners underwent otolith microchemistry analysis. **Results:** 1392 samples were successfully genotyped at 263 SNPs post-filtering (average read depth = 124.4; genotyping rate = 90.8%), including 726 spawners, 249 smolts, 281 pre-smolts, and 136 fry. 97% of smolts were genetically assigned sockeye. Furthermore, in line with prior findings, the majority of genetically-assigned hybrids exhibited no microchemical evidence of having spent time in a marine environment, including roughly half (48%) of backcross sockeye. Interestingly, anadromous and resident backcross sockeye exhibited significantly different morphology, with resident individuals being smaller. Stock proportions among spawners varied greatly from year to year, but over the time period of this study (2018-2023), there was a general increase in the proportion of hybrids.

Conclusions: Together, these data suggest that hybrids predominantly exhibit a resident life history. If maintaining a large *O. nerka* population is a management goal, then the low frequency of anadromous hybrids would support this. However, if maintaining the genetic integrity of sockeye and kokanee is a goal, then the increase in hybrids may warrant further management interventions, such as additional spawning channels targeted at a single migratory ecotype to attempt to limit hybridization. Overall, this study highlighted the ongoing fluctuations in stock proportions in the system, underscoring the need for continued monitoring with consistent sampling as the system progresses towards equilibrium.



Active predators do not necessarily specialize in sedentary prey: a simulation model

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POSTER SESSION 1 - **P01.343**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Predators exhibit diverse foraging modes, ranging from ambush to active pursuit of prey, each associated with specific benefits, costs, and prey preferences. While ambush predators are associated with capturing mobile prey, the specialization of active predators on sedentary prey, as suggested by key papers discussing the foraging mode paradigm, remains less understood. The presented study aimed to examine the circumstances under which active predators preferentially capture sedentary prey. Using a spatially explicit individual-based simulation model, I manipulated the spatial patterns of sedentary prey, the movement directionality and speed of mobile prey and active predators, and the presence of competing ambush predators. Key factors such as area-restricted search (ARS) by active predators, uncertain capture success of prey, and prey reappearance after capture were also considered. The simulation results suggest that active predators do not necessarily specialize in sedentary prey.

Instead, this tendency is influenced by prey spatial patterns and competition from ambush predators. Clumped spatial patterns of sedentary prey and the use of ARS by active predators increased encounters with sedentary prey, while competition with ambush predators drove active predators to focus on sedentary prey. However, non-directional movement by predators and faster-moving prey often resulted in a higher proportion of mobile prey being captured. These findings highlight the complexity of predator-prey interactions and challenge assumptions about active predator specialization. This study underscores the importance of integrating spatial and behavioral dynamics into predator-prey models, offering a framework for predicting ecological outcomes in diverse systems. Future research could refine these findings by incorporating additional variables such as habitat heterogeneity, energy costs, and learning.



Adaptive Laboratory Evolution of *Aspergillus niger* Using Single-Conidium Sorting for Enhanced Citric Acid Production

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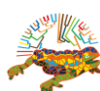
POSTER SESSION 3 - **P03.251**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Aspergillus niger is the primary organism used for industrial-scale production of citric acid, a widely used organic acid in food, pharmaceuticals, and cleaning products. As global demand increases, improving the productivity of *A. niger* strains is a key goal in biotechnology — supporting more efficient and environmentally sustainable bioprocesses. In applications where fungal biomass is reused, such as animal feed, non-GMO strain improvement is especially important to meet regulatory and consumer expectations.

Adaptive Laboratory Evolution (ALE) offers a powerful route to enhance complex traits like citric acid overproduction by applying long-term cultivation under defined selective pressures. This approach enables the gradual accumulation of beneficial mutations without direct genetic modification, making it well-suited for industrially relevant traits. However, ALE in filamentous fungi is often constrained by

population heterogeneity, genetic drift, and uneven growth rates.

To overcome these limitations, we incorporate flow cytometry-based sorting of single conidia into the ALE workflow. Forward scatter serves as a proxy for conidial size, which correlates with citric acid production and allows selective propagation of individuals with desirable phenotypes. This targeted approach enhances the evolutionary pressure toward desirable phenotypes while avoiding random bottlenecks and preserving genetic diversity. Applying environmental pressures such as insoluble nutrient sources (e.g. insoluble phosphate source) can promote adaptation toward higher citric acid production, as the acid aids in nutrient solubilization. Combined with genomic and transcriptomic analysis, this framework provides a precise, non-GMO strategy for evolving *A. niger* strains with enhanced citric acid productivity and direct suitability for industrial use.



Adaptive laboratory evolution of *Saccharomyces* and non-*Saccharomyces* wine strains under oxidative stress: phenotypic shifts and genomic perspectives

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¹Universitat Rovira i Virgili, ²Stockholm University

POSTER SESSION 3 - **P03.303**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

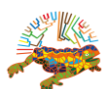
Reactive oxygen species (ROS), such as hydrogen peroxide (H₂O₂), can cause extensive damage to cellular macromolecules, ultimately leading to cell death. Yeast cells can mitigate oxidative stress by producing specific metabolites and antioxidants, which are particularly important traits for wine making. In this study, we used adaptive laboratory evolution (ALE) under oxidative stress conditions with populations of two commercial wine yeasts *S. cerevisiae* strains (Lalvin CLOS and Red Fruit) and two *Z. rouxii* strains (CW95 and CW96). Our main goal was to enhance their tolerance to oxidative stress and investigate associated phenotypic and genomic changes. Following an initial tolerance screening, strains were placed into individual batch cultures with an initial oxidative stress concentration of 12 mM of H₂O₂. Every five generations, part of each population was transferred to fresh media, maintaining or increasing oxidative stress based on growth kinetics improvement. After ~250 generations, evolved populations and individual clones (genotypes) were selected based on their improved growth under oxidative stress.

Selected variants were further evaluated in alcoholic fermentations to assess changes in fermentative performance and production of secondary metabolites with antioxidant properties, such as hydroxytyrosol. Whole-genome sequencing was conducted on populations sampled at intermediate generations and at the endpoint of ALE as well as on selected individual clones, to track the emergence and dynamics of de novo mutations. All evolved populations (*S. cerevisiae* and *Z. rouxii*) demonstrated improved oxidative stress tolerance, growing in

20 mM H₂O₂ with fitness comparable to non-stressed ancestral strains. However, selected evolved *S. cerevisiae* populations and individual clones showed slower fermentation rates compared to their ancestors, although most eventually completed sugar consumption. Notably, clones of *S. cerevisiae* Lalvin CLOS and *Z. rouxii* CW96 improved in hydroxytyrosol production relative to their ancestors.

Genome sequencing analysis yielded a wide array of de novo single-nucleotide polymorphisms (SNPs), which were annotated to identify their functional impacts. In the case of Lalvin CLOS strain 50 de novo SNPs were identified in the evolved population with highest oxidative stress tolerance. Of these, few SNPs, mostly on chromosomes XIV and XVI reached near-complete fixation (allele frequency ~0.9). Tracking allele frequency trajectories revealed early emergence and fixation of some mutations, while others appeared transiently or declined over time. Interestingly, we observed distinct evolutionary patterns between the *S. cerevisiae* strains. Lalvin CLOS replicates followed parallel evolutionary trajectories with multiple identical SNPs across all populations. In contrast, Red Fruit strain displayed a divergent evolutionary path with each replicate acquiring a more unique set of SNPs than shared with the other replicates.

Our results demonstrate that ALE is an effective approach to improving oxidative stress resistance in wine yeast strains, though it may impact fermentative performances. Our work provides phenotypic and genomic insights into the diverse adaptive responses that yeast can exhibit under long-term oxidative stress exposure.



Adaptive physiological responses of *Daphnia pulicaria* to elevated salinity

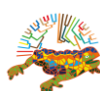
Paida V¹, Zeis B², Isanta-Navarro J¹

¹Freshwater Biology Section, University Of Copenhagen, ²Institute of Integrative Cell Biology and Physiology (IIZP), University of Münster

POSTER SESSION 1 - P01.151, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Freshwater ecosystems all over the world are under severe threat from a number of anthropogenic stressors, including climate change, invasive species and salinization. Therefore, the capacity of species to rapidly adapt to altered environmental conditions is critical for their prevalence. Resurrection ecology studies explore evolutionary responses of natural *Daphnia* populations to stressors by hatching dormant eggs from different periods with distinct environmental conditions. A recent resurrection ecology study in Tanners Lake (USA) showed that the latest subpopulations of the keystone herbivore *Daphnia pulicaria* are resilient to increased salinity. While the improved survival rates and the genomic data provide an indication of the adaptation, we studied

the effects of salinization on relevant phenotypic traits to capture the complex organismal responses through time. For example, increased salinity directly affects physiological processes, such as metabolic rate and lipid peroxidation levels, in populations that have not been exposed to this stressor before. Hence, we measured hemoglobin, as a proxy for oxygen delivery to the tissues and thiobarbituric acid reactive substances (TBARS), which is an indicator of lipid peroxidation, to provide insights into the adaptation mechanisms of the resurrected populations. Our results indicate that the adaptation to salinization of *Daphnia* over decades is partially due to a remodeled physiology, and a resulting reduced metabolic cost.



Adaptive significance of the effects of population density on life history traits in a moth

Kuusk K¹, Esperk T¹, Teder T¹, Holm S², Tammaru T¹

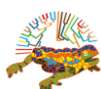
¹University of Tartu, ²Swedish University of Agricultural Sciences

POSTER SESSION 3 - **P03.329**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Larval density is a key determinant of insect life history traits, influencing a broad spectrum of fitness-related characteristics, including body size, development duration, fecundity, immune responses, and coloration. Despite significant advances, a comprehensive understanding of the ultimate causes, underlying mechanisms, and evolutionary implications of density-dependent effects in insects remain incomplete. To more precisely characterize the effects of larval density and assess the sensory capabilities of lepidopteran larvae in distinguishing conspecifics from heterospecifics, we recorded the life history traits in the geometrid moth, *Hypomecis atomaria*, larvae reared under three experimental conditions. These conditions included rearing the larvae with (1) conspecifics, (2) larvae of three other lepidopteran species, and (3) either larval or adult stages of a dipteran, included to broaden the taxonomic scope of our design. That was done to determine whether larvae predominantly perceive density-associated signals through visual, mechanical, or tactile

cues. As response variables, we measured pupal mass and larval development time in our focal species.

Our results indicate that increased density of conspecifics in *H. atomaria* is associated with a shortened larval development period and reduced pupal size when compared to individuals reared in isolation. We observed similar, albeit weaker, responses to the presence of heterospecific lepidopteran larvae, while no significant effects were detected in response to dipteran larvae. No single signal examined demonstrated statistically significant effect in eliciting a crowding response. Moreover, our findings reveal that crowding effects in *H. atomaria* are gradual, with the magnitude of the response varying depending on the nature of the cue present. These findings suggest that larval density has a distinct impact on the life history traits of *H. atomaria*, and that the underlying mechanisms may reflect an intermediate response between a reaction to conspecifics and a more generalized reaction to environmental disturbance.



Are fish on land to escape an intolerable aquatic environment?

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POSTER SESSION 2 - P02.005, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Colonisations of novel habitats are important events in evolution, but the factors that initially prompt such ecological transitions are often unknown. The invasion of land by fish represents an extreme habitat transition that offers an ideal opportunity to empirically investigate the causes of major ecological transitions. The intertidal ecotone—and rock pools in particular—have been an important staging ground for terrestrialization. Classic hypotheses focus on the adverse abiotic conditions of rock pools at low tide as instigators of fish voluntarily stranding themselves out of water, which can then lead to the evolution of an amphibious lifestyle. To test these hypotheses, we studied the abiotic conditions of rock pools on the island of Guam, where various species of aquatic,

amphibious, and terrestrial blenny fishes exist. We found little support for the expected deterioration of abiotic conditions in standing pools at low tide (salinity, pH, and oxygen), and fish were not seen to be excluded from those pools. Rock pools were found to have oxygen levels within the requirements of most blennies and those of other marine fishes reported in the literature. We conclude that the abiotic environment of rock pools alone was unlikely to have instigated the evolution of amphibious behaviour in blennies, at least on Guam. Instead, the broad range of abiotic conditions experienced in rock pools suggests these conditions could have primed amphibious blennies to better endure the novel conditions on land.



Assessing genotype imputation methods for low-coverage sequencing data in populations with differing relatedness and inbreeding levels

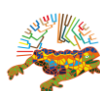
Vi T¹, **Lloret-villas A**², Santure A¹

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POSTER SESSION 3 - P03.337, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Low-coverage sequencing (LCS) followed by genotype imputation has become a cost-efficient approach for obtaining whole-genome SNPs. Several imputation methods for LCS data have been developed over the last decade. However, comparisons of their accuracy in inferring missing genotypes and their effectiveness for downstream analysis such as population genetics have not been comprehensively studied. In the present study, we assessed imputation performance of five different tools: GLIMPSE2, GenImp, QUILT2, STITCH, and Beagle5.4, using populations simulated by SLiM4 that represent different levels of genetic relatedness and inbreeding. Imputation accuracy was calculated at the level of variant, haplotype, and sample. The

effectiveness of using imputed genotypes in recovering genetic structure, relatedness, inbreeding coefficients, and demographic history was subsequently evaluated. The imputation accuracy of different methods was further tested in a real population of 283 hihi (stitchbird) samples. Our results suggest a high accuracy of all the tested methods on populations with high levels of genetic relatedness. However, imputation accuracy of different tools and the results of downstream analysis were affected differently across other scenarios of population structure. The simulation and imputation pipeline presented here can help determine the most suitable imputation method for different population scenarios.



Assessment of Transparency Across Articles in Ecology and Evolutionary Biology Journals, 2000–2024

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POSTER SESSION 3 - P03.357, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

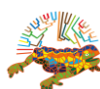
Background: Ecology and evolutionary biology (EcoEvo) research plays a vital role in evaluating and addressing environmental challenges. Transparent reporting — through study registration, conflict of interest (COI) and funding disclosures, and sharing of data and code — is essential for enhancing the credibility, impact, and reproducibility of ecological research. However, little is known about the status and trends of research transparency across EcoEvo literature. To address this gap, we conducted a systematic assessment of transparency indicators in a random sample of 625 original research articles published between 2000 and 2024 across 231 EcoEvo journals.

Materials and Methods: On January 31, 2025, we retrieved metadata for 443,655 English-language research articles published between 2000 and 2024 in 231 journals classified under ‘Ecology’ and ‘Evolutionary biology’ in the Web of Science Journal Citation Reports. From this dataset, we randomly selected 25 original research articles per year, resulting in a final sample of 625 articles with data and analyses. Three independent reviewers screened the full text to identify statements related to study registrations, COI and funding disclosures, and publicly available datasets and code. For each indicator, we documented the exact statement and additional information (e.g., funding source, location of data sharing such as repository or supplementary information) using a standardized protocol (<https://osf.io/bn5wp/>).

Results: Across the 625 EcoEvo articles, none included statements related to study registration. A total of 147 (23.5%) articles included COI disclosures, with nearly all (144; 98.0%) reporting no conflicts of interest. Funding information was provided in 539 (86.2%) articles, with most reporting public (261; 48.4%) or a

combination of public and non-governmental organization (194; 36.0%) funding sources. There were 177 (28.3%) articles that discussed some level of publicly shared data sets. Of these, 150 (24.0%) shared data via public repositories or supplementary materials; 21 (3.4%) indicated availability upon request. Although three articles (0.5%) claimed data were shared, no dataset could be located. Among the 611 articles where code availability would be relevant, excluding articles that, e.g., used descriptive morphological comparisons or qualitative interviews, we detected 46 (7.5%) articles with a code availability statement. Among articles that included a code availability statement, 36 (88.1%) shared code via a repository, five (11.9%) shared code using supplementary materials, four (9.5%) shared code upon request, and one (0.2%) explicitly stated that code was not shared. From 2000 to 2024, the proportion of articles with conflict of interest disclosures increased from 0.0% (95% CI, 0.0%–13.3%) to 88.0% (95% CI, 70.0%–95.8%), while funding disclosures rose from 80.0% (95% CI, 60.9%–91.1%) to 92.0% (95% CI, 75.0%–97.8%). The proportion of articles with data sharing increased from 8.0% (95% CI, 2.2%–25.0%) to 64.0% (95% CI, 44.5%–79.8%), and code sharing rose from 0.0% (95% CI, 0.0%–13.3%) to 32.0% (95% CI, 17.2%–51.6%).

Conclusions: Over the past two decades, transparency in EcoEvo research has improved, particularly with regard to COI and funding disclosures and data sharing statements. However, the absence of study registration and persistently low levels of code sharing in our sample highlight areas for improvement. Together, these practices increase the credibility and long-term value of EcoEvo research by making results more verifiable and reusable.



Associative Learning in Plants

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POSTER SESSION 3 - **P03.298**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

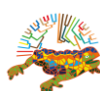
Background: Associative learning is a widespread trait in the animal kingdom because of the significant selective advantage it provides, and has even been documented in some non-neural organisms, such as certain species of amoebae. However, although plants are known to exhibit memory, environmental perception, and adaptive behavior, their capacity for associative learning is still largely unexplored, and the few existing studies about the topic have yielded inconsistent or controversial results. This study investigated the ability of the annual legume *Phaseolus vulgaris* (common bean) to form associations between light as an honest stimulus and vibrations as a neutral one.

Materials and methods: *P. vulgaris* was selected as the model species due to its upwards photonastic leaf movements in response to light, which provide a clear, trackable response. Over a 12 day training phase, the plants were subjected to either an association treatment, in which light presentations were paired with motor-controlled soil vibrations; or a control treatment, in which the light presentations were unpaired with the vibration periods.

After the training phase, the plants underwent a day-long testing session where only soil vibrations were presented, without light. Throughout the experiment, the plants were continuously photographed, and the pictures were used to analyze leaf movements with the Social LEAP Estimates Animal Poses (SLEAP) software.

Results: In the testing phase, plants from the association group exhibited significantly higher leaf angles compared to those in the control group. However, this response was not correlated with the occurrence of the vibration stimulus.

Conclusions: The lack of a specific response to vibrations limits the interpretation of our findings as evidence of associative learning. Nevertheless, the results indicate that *P. vulgaris* may exhibit a general anticipatory response following training with paired stimulation. This behavioral shift, although not definitively associative, merits further investigation. Currently, research with refined experimental conditions is being conducted to expand on these findings and determine the possibility for associative learning in *P. vulgaris*.



Being plastic is fantastic? How climate influences morphology across developmental strategies in European salamanders and newts

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POSTER SESSION 3 - **P03.256**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Climate is an important driver of morphological variation as it imposes substantial selective pressures on organisms, shaping the energetic demands, survival strategies, and locomotor or thermoregulatory adaptations of organisms. This is particularly true for ectothermic species, which cannot produce metabolic heat and thus rely heavily on environmental temperatures to regulate their physiological processes. Among amphibians, salamanders and newts are highly sensitive to environmental conditions due to their permeable skin and dependence on moist habitats. Changes in size or shape can affect their gas exchange ability and therefore their survival in drier or hotter environments. This study explores how climatic factors - such as temperature, precipitation, humidity, and seasonality - influence external morphology and bone shapes across populations of two widely distributed European species with facultative life cycle strategies, the alpine newt (*Ichthyosaura alpestris*) and the fire salamander (*Salamandra salamandra*). These species possess alternative developmental strategies (complex and simple) which may modulate morphological responses to environmental changes. Fire salamander can be larviparous (complex life cycle), giving birth to numerous aquatic larvae, while pueriparous females (simple life cycle) produce fewer, fully metamorphosed terrestrial juveniles. The alpine newt can follow a triphasic life cycle (complex life cycle), with an aquatic larval

stage, a terrestrial juvenile stage, and a terrestrial adult stage—although adults return to the water during the breeding season. Some other populations can be paedomorphic (simple life cycle), reaching sexual maturity while retaining larval characteristics remaining fully aquatic throughout their lives. Because populations with a complex life cycle can produce multiple morphologies to transition between environments during ontogeny, we hypothesize that this plasticity enhances their ability to adapt to a wider range of climatic conditions. Consequently, they should exhibit more pronounced climate-driven morphological shifts than populations with a simple life cycle. Here, we combined linear measurements and geometric morphometric data, to quantify respectively the external morphology and bone shape, of 1600 individuals belonging to 150 populations with different life cycle strategies, with climatic variables across geographic gradients, we aim to identify the impact of environmental conditions on morphological patterns and life cycle plasticity. Preliminary results suggest that climate, particularly seasonality and humidity, significantly affect morphology. Distinct morphological patterns emerge between individuals with simple versus complex life cycles in these two species, highlighting the importance of considering both environmental and developmental contexts when examining morphological evolution in amphibians and underscore the vulnerability of these taxa to ongoing climatic change.



Beyond Genetic Analogy: Variance Proportionality in Cultural Evolution

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POSTER SESSION 3 - P03.314, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The formalization of trait transmission and diversification remains an open question for cultural evolution. Although concepts from evolutionary biology often guide cultural evolutionary research, there has yet to be a robust analogue to Fisher's variance decomposition—an essential tool in evolutionary genetics that partitions phenotypic variance into distinct genetic components. Classical cultural evolutionary models frequently represent cultural traits discretely, similar to genetic alleles, despite significant conceptual limitations in this analogy. We advocate for a shift towards conceptualizing cultural traits as continuous points within multidimensional spaces, paralleling vector-based representations used widely in evolutionary morphometrics and machine learning.

In biological inheritance, offspring trait variance is typically modeled as constant, unaffected by the variability between parents due to the discrete, particulate nature of genetic transmission. This is because every chance event happens on the individual level. Several schools of study of cultural evolution, such as memetics, arbitrarily adapted this paradigm for culture. In contrast, we hypothesize that cultural inheritance fundamentally differs, resulting in offspring variance that scales proportionally with the variance among cultural models (or "cultural parents"). We suggest that this variance proportionality in cultural transmission arises through two evolutionary mechanisms unique to cultural systems that necessarily integrate information from multiple sources which lack the clear genotype-phenotype distinction. First, inherent cognitive constraints in accurately estimating averages from diverse parental inputs (error variance); and second, intentional exploration proportional to the diversity of successful strategies (exploratory variance).

To empirically test this hypothesis, we conducted controlled transmission-chain experiments where participants undertook an 'averaging task'. Participants were provided with multiple digitally generated 3D-printed 'parental' artifacts and asked to produce offspring artifacts that represented an

average of these samples. This experimental setup allowed us to precisely measure the variance of parental inputs. The positions of these offspring artifacts were then analyzed as vectors within the multidimensional trait space, allowing for the measurement of distance from parental mean and demonstrating offspring variance proportional to parental variance.

These findings are supported by the results of our previous related studies. In one, participants were asked to estimate the midpoint between two spatial points; results indicated that greater distances between points made accurate midpoint estimation significantly more challenging. Another study involved participants estimating numerical answers with provided "hints" in the form of previous participant answers; findings showed that increased variance among the provided hints resulted in greater variance among participants' answers.

The implications of variance proportionality in cultural transmission are substantial. It might explain why cultural evolution produces rapid, punctuated changes amid periods of cultural stasis, or why the sympatric cultural divergence is relatively common, while the homologous sympatric speciation is considered a rare and slow phenomenon in genetic evolution. This might offer an explanation for the ubiquity and frequency of clearly delineated subcultures within human populations without requiring geographic isolation or disruptive selective forces.

Our findings suggest that cultural inheritance operates under fundamentally different constraints than genetic inheritance. With the approach presented here, we aim to offer evolutionary biologists a conceptual toolset adapted to understanding dynamics resulting from proportional variance. If similar processes of information integration from multiple sources give rise to stochastic terms in the biological domain, the findings from cultural evolution can be easily generalized and applied in the field of organismal evolution.



Biogeographic resilience in Patagonian spiders: Glacial refugia and distribution shifts of *Philisca* species during Pleistocene climatic oscillations

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POSTER SESSION 3 - P03.318, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Over the past millions of years, glaciations and associated climate change, volcanism, and marine transgressions have drastically altered the geography and biodiversity of Patagonia. The numerous advances and retreats of glaciers during the Pleistocene, such as the Last Glacial Maximum (LGM) and the Last Interglacial Period (LIG), greatly modified the region's landscape. Previous studies have identified glacial refugia in Patagonia for vertebrates and vascular plants, some of which are located in the Valdivian Forest. Defining these climatic refugia during adverse periods is of great interest, as they were crucial for the survival of various taxa.

In this dynamic region, spiders of the genus *Philisca* have undergone significant diversification over the last 18 million years, adapting to the dramatic climatic and geological shifts that shaped Patagonia. The species of this genus primarily inhabit the Valdivian Forest in Argentina and Chile. *Philisca accentifera* and *Philisca atrata* are particularly notable due to their broad distributions spanning both sides of the Andes. The aim of this study is twofold: (1) to reconstruct the paleo distribution of *P. atrata* and *P. accentifera* during the LGM and the LIG using species distribution modeling, and (2) to identify potential glacial refugia that may have been critical for their persistence during Pleistocene climatic extremes using genomic data.

For the modelling aspect of this project we conducted a search for all occurrence records of the involved species with data sourced from museum collections. Additionally, we included records from field collections carried out by the authors between 2021 and 2024. Climatic variables related to humidity and temperature, used to construct present-day distribution models, were obtained from the CHELSA 2.0 repository. Models

for each species were built using a combination of different algorithms. An ensemble of the models generated by these algorithms was created for each species. Finally, the present-day models were extrapolated into the past (LGM and LIG) using the same climatic variables but with reconstructions available in the PaleoClim repository. Regarding the molecular aspects of this work, individuals from the tissue collection from the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" were used as well as tissue taken from the individuals collected from 2021 to 2024 and preserved on ethanol 96% at -20°C. DNA was extracted and purified using magnetic nanoparticles (developed by Universidad Nacional de La Plata) and QIAGEN Blood and tissue kits. Genomic libraries were prepared following Etter protocol (with modification) and were sequenced on an Illumina NovaSeq X in 150 bp paired-end mode at Macrogen Inc. (Seoul, South Korea).

We observed that the distributions of the *Philisca* species included in the analysis during the LIG were similar to their current distributions, though slightly broader. However, during the LGM, a strong restriction in potential distributions was evident for the two species. These last reconstructions include areas of high suitability during the LGM coincident with a lowland refuge and a peripheral refuge previously defined for vascular plants and vertebrates.

Thus, our results suggest that the studied species may have experienced fluctuations in their distribution ranges throughout their evolution over the past 130,000 years. These findings not only reveal how climate change shaped spider biogeography in Patagonia, but also highlight the Valdivian Forest as a persistent biodiversity sanctuary through glacial cycles for a group of invertebrates.



Biogeography of Southeast Asian Melastomes - insights from a phylogenomic study of Dissochaeteae

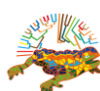
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POSTER SESSION 3 - P03.276, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Melastomataceae is one of the largest and most diverse angiosperm families in the tropics. However, despite their high diversity and ecological significance, many Southeast Asian lineages remain understudied. The tribe Dissochaeteae comprises c. 70 spp. of lianas and shrubs distributed primarily in primary and secondary forests across tropical Asia. We present a comprehensively sampled phylogenomic framework of the tribe utilizing high-throughput sequencing data. This serves as a foundation for

understanding the historical biogeography of Dissochaeteae, shedding light on patterns of diversification and dispersal across Southeast Asia. The tribe seems to follow a pattern of multiple and relatively rapid West to East dispersals in Southeast Asia. Furthermore, we are working towards comparing phylogenetic patterns across multiple melastome lineages within the region, thereby contributing to the broader discussions on the (bio)geographic history of the region's flora.



Biologically inspired warning patterns deter birds from wind turbines

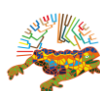
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POSTER SESSION 3 - **P03.252**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Wind power plants have been at the forefront of investment and innovation in renewable energy. However, bird fatalities from collisions with wind turbines present an ecological and social challenge to the growing deployment of wind power. Increasingly, research has focused on utilizing the sensory ecology of animals to provide passive or active cues that minimize collision risk by increasing the detectability and/or aversiveness of the turbines. In nature, numerous aposematic species use contrasting colors and striped patterns to warn birds of their unpalatability. These common signal elements are effective due to their salience across natural scenes, memorability, generalizability across taxa due to mimicry, and exploitation of the innate color preferences of birds. This begs the question: might biologically inspired turbine blades that mimic aposematic patterns help

to protect birds by increasing their avoidance of the blades? Here, we use a screen-based 'bird game' experimental setup to test the behavioral responses of wild-caught great tits (*Parus major*) to three blade patterns currently used for wind turbines (white, red striped, and single black blade) as well as a novel biologically inspired aposematic pattern. Birds took significantly longer and were less likely to approach patterned compared to uniform white blades irrespective of whether they had prior experience with them. This effect was strongest for the aposematic pattern compared to all other patterns tested, highlighting the utility of our bio-inspired approach. Our work suggests that adding red, black and yellow warning patterns to wind turbine blades will likely reduce the risk of bird collisions.



Birdlife in the Anthropocene: Understanding convergent adaptation to urbanization

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POSTER SESSION 3 - P03.316, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The rapid onset of urbanisation is inflicting dramatic changes on the natural world, presenting an unparalleled evolutionary challenge. Currently, 46% of the global human population resides in urban areas, a figure that continues to rise. Understanding the impact this has on biodiversity is challenging due to the complex and often unpredictable interactions between organisms and their rapidly changing environments. Birds, with their exceptional adaptability, serve as a particularly useful model for studying the effects of urbanisation on wildlife.

Here, we investigate how bird species have adapted to urban environments through a comprehensive analysis of phenotypic and genomic data. By combining big data sources such as the citizen science project eBird, alongside anthropogenic metrics like light pollution and population density, we have produced estimates of urban association across the avian tree of life. Integrating these with life trait data from available datasets such as AVONET, our main aim is to identify traits that predict urban tolerance and that facilitate

transitions to anthrodependent niches at the macroevolutionary scale.

Focusing specifically on the *Passer* genus, renowned for its urban adaptability, we additionally performed a comparative study of urban and rural populations of the tree and house sparrows in Europe and Asia, where both species are highly associated with human environments. By examining morphological, behavioural, and genomic data across these populations and of other species within the genus, we seek to identify convergent signatures of selection and adaptation to urban environments at a more fine-grained scale. While we observe trait variation between urban-associated and less associated species, population structure within *Passer* is not distinctly divided between urban and rural environments.

This research enhances our understanding of how urban environments shape the evolution of bird species, revealing what adaptations promote survival in these modified habitats. These insights can inform conservation strategies designed to protect urban biodiversity and mitigate the impacts of urban expansion on wildlife.



Building Bridges in Evolution Education: A Teacher Training Experience in Argentina

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POSTER SESSION 3 - P03.316, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The teaching of evolutionary biology in Argentina faces significant challenges, including limited access to formal training for educators, especially those outside major urban centers. In response, we organized “Tras las Huellas de la Evolución: construyendo una mirada crítica de la evolución en el aula”, a week-long teacher training course for teachers from different parts of the country held in July 2023 at the Universidad de Buenos Aires. Funded by ESEB’s Global Evolutionary Biology Initiative (GEBI), this project aimed to strengthen evolutionary education by providing interdisciplinary training, fostering collaboration between educators and researchers, and creating accessible teaching resources.

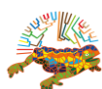
The course brought together 36 secondary and university-level teachers from across Argentina, with particular emphasis on participants from underserved regions. Thanks to financial support covering travel and accommodation, we ensured representation from diverse geographic areas that typically lack access to such training opportunities. The curriculum was carefully designed around four interconnected themes that addressed both foundational evolutionary concepts and their practical classroom application. Beginning with the (1) History of Evolutionary theme, sessions traced the development of scientific ideas while examining how controversies shaped our current understanding. (2) The Teaching Evolution theme went beyond theory, offering concrete strategies to work with common conceptions like teleology. (3) The broaden perspectives theme, interdisciplinary approaches connected evolution to other fields, from deep-time visualization activities to critical discussions about reductionism in health sciences. (4) The Human Evolution module brought these concepts to life through examination of 3D-printed hominid skulls, while maintaining constant focus on pedagogical applications. Throughout all sessions, participants collaborated to develop practical lesson plans under the guidance of biologists, historians, and researchers in science

education, resulting in a valuable collection of resources later shared through a public digital repository.

The designed lesson plans, compiled into a digital repository, were showcased at a symposium during the V Argentinian Reunion of Evolutionary Biology (RABE). This event strengthened ties between educators and researchers, with 35 out of 36 participants completing the program. Feedback underscored the course’s success in building networks and empowering teachers to tackle evolutionary concepts confidently.

Despite challenges—including Argentina’s ongoing economic crisis, which threatens to curtail similar future initiatives—the project demonstrated the transformative potential of targeted teacher training. Its success underscored how accessibility hinges on thoughtful support structures: GEBI’s funding, coupled with institutional resources like university dormitories and pro-bono instructors, proved critical in enabling participation from marginalized regions. Equally vital was the interdisciplinary collaboration that shaped the course, where diverse academic backgrounds—from biology to anthropology—fueled richer discussions and more nuanced pedagogical strategies. Beyond the immediate impact, the initiative prioritized sustainability through openly shared lesson plans and future plans for video-based resources, ensuring its reach extends far beyond the original participants. Together, these elements not only amplified the project’s impact but also modeled how such programs might thrive even in resource-limited contexts.

This experience highlights the urgent need for scalable, context-sensitive evolution education programs in Latin America, where socioeconomic disparities often limit professional development of teachers. By uniting researchers, educators, and international funding bodies, we can bridge gaps in science literacy and inspire evidence-based teaching.



Butterfly adaptations to seasonal tropical environments: links between, polyphenism, reproductive diapause, and migrations

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POSTER SESSION 1 - **P01.177**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Most tropical regions experience seasonal variation in rainfall, with varying degrees of seasonal variation in temperature. These conditions pose severe challenges to animals such as butterflies that depend on plant growth for reproduction, and on plant reproduction for sustenance (flower nectar or fruits). A conspicuous adaptation to this seasonality is seasonal polyphenism. Tropical satyrine butterflies often show wet-season forms with colour patterns with eyespots that help them survive attacks during activity, while dry-season forms have cryptic colorations that prevent attacks while at rest. To achieve seasonal polyphenism that fits the local environment, populations from regions that differ in relationships between rainfall and temperature can be expected to respond differently to environmental cues. Seasonal reproductive diapause and migrations are further strategies that are less conspicuous.

We investigated how climate is linked to developmental plasticity underlying seasonal polyphenism, reproductive diapause, and seasonal use of forest and savannah habitats, in the common evening brown *Melanitis leda* (Nymphalidae: Satyrinae). We performed common garden experiments to detect differences in responses to cues among populations from different climates. We also performed monthly butterfly sampling in various parts of the old-world tropics to document abundance, seasonal phenotypes, and the reproductive status of females.

The common garden experiments showed that *M. leda* populations from different parts of Africa, and wet and dry sides of the Western Ghats (mountains) in India, respond differently to rearing temperature and host plant species. We found that in regions with severe dry seasons, butterflies tend to remain unmated during dry seasons and are thus in reproductive diapause, while they can remain reproductively active where the dry season is less pronounced. We also discovered that *M. leda* and several other species are mainly found in the savannah during the wet season and mainly in the forest during the dry season, indicating a seasonal habitat shift not considered before. Our results indicate that the seasonal forms must not only fit with butterfly activity patterns and seasonally changing background colours and predator communities, but also to seasonal migrations between habitats. *M. leda* and other species might rely on landscapes with both forest and savannah, but the spatial scale of these migrations remains unclear. The differences between populations showed that *M. leda* can adapt to local climates even across populations that are only hundreds of kilometers apart. Overall, these butterflies have evolved a suite of traits to thrive in seasonal tropical environments, including locally adapted seasonal polyphenism, reproductive diapause, and seasonal habitat shifts.



Characters changing speed: the Covariomorph model and its impact on phylogenetic trees

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POSTER SESSION 1 - P01.049, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The rate of evolution of a single morphological character is not homogeneous across the phylogeny. In other words, morphological characters often show more lability in one clade than others. Furthermore, this rate heterogeneity varies between morphological characters. However, traditional models of character evolution for morphological datasets often assume that all characters evolve according to a homogeneous Markov process which applies uniformly across the entire phylogeny, potentially oversimplifying the complex dynamics underlying trait evolution. While models incorporating among-character rate variation alleviate the assumption of the same rate for all characters, they still fail to address lineage-specific rate variation for individual characters.

To address this limitation, we applied the covarion model, originally developed for molecular data to model the invariability of some sites for part of the phylogeny. The covarion model provides a promising framework for addressing the issue of homogeneous rates of evolution for characters in different lineages in morphological phylogenetics. In this study, we extend the covarion model in RevBayes to morphological character evolution, which we call ‘covariomorph’, and apply it to a diverse range of morphological datasets. Our covariomorph model utilizes multiple rate categories derived from a discretized probability distribution, which scales rate matrices accordingly. Characters are allowed to evolve within any of these rate categories, with the possibility of switching between rate

categories during the evolutionary process. This model of rate variation implies that different selective pressures act on different characters across the phylogeny, thereby enabling a more flexible and realistic representation of morphological evolution.

We verified our implementation of the covariomorph model with the help of simulations and also established connections of this model with the standard models of morphological evolution. Additionally, we examined nearly 200 published datasets, finding covarion-like rate variation in approximately half of them. Upon further examination of some of the selected datasets, we found that the covariomorph model provides a more nuanced approach to incorporate rate variation across lineages, significantly affecting the resulting tree topology compared to traditional models. Additionally, we observed notable differences in the inferred branch lengths between the covariomorph model and conventional models.

Our findings demonstrate that the covariomorph model enhances our ability to accommodate rate variation across morphological characters, further impacting the topological inference of the phylogenetic tree and its temporal aspects. The observed sensitivity of branch lengths to model choice underscores potential implications of this approach for divergence time estimation and evolutionary rate calculations. We conclude that the covariomorph model’s capacity to incorporate complex dynamics found in empirical data strengthens phylogenetic inferences using morphological characters.



Chromosome-Level Genome Assemblies Across Five Brassicaceae Species: New Resources for Comparative Evolutionary Studies

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POSTER SESSION 3 - P03.294, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The Brassicaceae family includes economically important crops and ecological model species, yet many of its lineages remain underrepresented in genome-scale studies. Despite progress in assembling reference genomes for a few Brassicaceae species, detailed insights into lineage-specific genome evolution, structural rearrangements, and repetitive DNA dynamics are still lacking. To address this gap, we generated high-quality, chromosome-scale genome assemblies for five representative species spanning distinct Brassicaceae species; *Aethionema saxatile*, *Cardamine glauca*, *Erysimum linariifolium*, *Noccaea praecox*, and *Odontarrhena muralis*. Using PacBio HiFi long-read sequencing and Hi-C scaffolding, we achieved chromosome-level assemblies

with high contiguity. Quality assessments indicated strong completeness, with BUSCO scores exceeding 95% and Merqury completeness values ranging from 78.15% to 96.67%. The assembled plastid genomes exhibit the conserved quadripartite structure typical of land plant plastomes, supporting their structural integrity. These genome assemblies provide valuable resources for the Brassicaceae research community, enabling future investigations into genome evolution, gene family dynamics, and structural variation across diverse lineages. As an initial application, we plan to conduct gene family expansion and contraction analyses to investigate lineage-specific adaptations and the evolutionary dynamics of gene content across these tribes.



Chromosome-level genome assembly and annotation of *Salvelinus malma*

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POSTER SESSION 3 - P03.222, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Salvelinus malma, belonging to the Salmonidae family, is an a critically endangered species in the China. To support genetic research and strengthen the protection of wild fish resources, the first chromosome-level reference genome of *S. malma* generated through integrated technologies from PacBio and Hi-C sequencing platforms. The genome

assembly has a total length of 2.52 Gb with contigs assigned to 42 chromosomes. A total of 45,385 protein-coding genes were annotated with excellent completeness (BUSCO evaluation: 98.8%). The chromosome-level genome of *S. malma* will serve as a valuable resource for future functional genomics research, while greatly facilitating its exploration and conservation.



Chronic heat tolerance reveals overestimated thermal safety margins and increased vulnerability in marine fish populations

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POSTER SESSION 3 - P03.289, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Predicting species' vulnerability to global warming is a central goal in evolutionary and physiological ecology. In marine fishes, ongoing changes in distribution contrast with their apparent capacity to tolerate temperatures ranging from 5°C to 25°C higher than current conditions. Using a dataset of 786 upper critical temperatures across 213 species and recent theoretical developments, we provide conclusive evidence that these so-called 'thermal safety margins' overestimate resilience to warming and that most species inhabit thermal conditions approaching their physiological tolerance limits. This result holds across latitudes, and historical records show that several populations have encountered stressful temperatures in the recent past. While warming tolerance remains similar across geographic regions, behavioral responses are constrained at lower latitudes, as the distribution shifts required to encounter cooler waters are disproportionately higher in the tropics. Chronic heat tolerance and the maximum environmental temperature experienced by

organisms exhibit significant phylogenetic signal, indicating that closely related species tend to share similar thermal traits. Notably, species currently listed as threatened by the IUCN tend to exhibit narrower thermal safety margins, suggesting that rising temperatures may interact synergistically with other stressors, such as overfishing or habitat degradation—particularly in vulnerable lineages like sawfish (Pristiformes) and sturgeons (Acipenseriformes). These findings gain greater significance in light of recent evidence that phenotypic plasticity may not fully compensate for evolutionary differences in heat tolerance across fish species, potentially limiting their ability to respond to rapid warming. This underscores the crucial role of evolutionary potential in determining species' long-term resilience, as plastic responses alone may fall short in buffering the impacts of accelerated climate change. Overall, our results illustrate how thermotolerance measures can be extrapolated to the field and used to quantify species' vulnerability to ongoing climate change.



Co-diversification of sperm and female sperm storage organs cause reproductive isolation in diverging populations

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POSTER SESSION 1 - **P01.271**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Understanding how reproductive traits evolve to create barriers to gene flow is central to speciation research. While much attention has focused on premating traits and species-level differences, a critical but rarely tested prediction is that traits enhancing fertilization success within populations may simultaneously reduce compatibility between diverging populations. Furthermore, most studies overlook the potential for postmating sexual selection to drive early reproductive isolation through interactions between male and female reproductive traits.

Here, we test whether divergence in two sexually selected traits—sperm length and the length of the female sperm storage organ (the seminal receptacle, SR)—can lead to postmating prezygotic (PMPZ) isolation in the early stages of population divergence. We use *Drosophila montana*, a species with naturally diverging populations that vary in the presence and strength of PMPZ isolation. First, we measured sperm and SR lengths across populations and found that incompatible population pairs (e.g., Colorado × Vancouver) exhibited greater divergence in these traits than compatible pairs. We also observed a strong positive correlation between sperm and SR length across populations, indicating co-diversification consistent with sexual selection. Within the Colorado population, natural variation in trait alignment predicted fertilization success: mismatches between sperm and SR length resulted in significantly reduced fertilization success.

To directly test the causal role of these traits in reproductive isolation, we experimentally

manipulated sperm and SR length through condition-dependent plasticity by rearing flies under high- or low-resource developmental conditions. Trait exaggeration (longer sperm and SRs) occurred only in the Colorado population, allowing us to isolate the effects of trait variation on fertilization outcomes. As predicted, mismatched trait values reduced fertilization success both within populations and in crosses between Colorado and Vancouver.

Our results show that sperm and SR lengths—both sexually selected traits in *Drosophila*—function as an ornament and signal receiver pair, codiversifying among populations and creating fertilization bias via postcopulatory sexual selection. When these traits diverge across populations, they contribute to PMPZ isolation, particularly in asymmetric patterns where fertilization fails in one cross direction but not the other. Moreover, our findings suggest that condition-dependent trait expression may amplify or mitigate isolation depending on environmental context.

Together, these results provide rare experimental support for the idea that postmating sexual selection can drive reproductive isolation through co-diversification of interacting traits. By linking within-population fertilization success to between-population incompatibility, our study identifies sperm and SR length as speciation phenotypes—traits that both evolve under sexual selection and contribute directly to the evolution of reproductive barriers.



Co-Evolution between Phages and Bacterial Pangenomes in the Light of Horizontal Gene Transfer

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POSTER SESSION 3 - P03.262, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Understanding the complex interactions between phages and their bacterial hosts is of considerable interest.

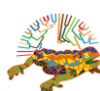
Understanding the co-evolutionary dynamics between bacteriophages and their bacterial hosts requires a detailed view of how accessory genes, shaped by horizontal gene transfer (HGT), influence phage infectivity. Bacterial pangenomes are highly dynamic, containing many mobile genes that can be gained or lost across strains and species. To dissect gene-level interactions underlying phage susceptibility and resistance, we present Goldfinder, a phylogenetically informed method to identify gene co-occurrence and co-avoidance patterns across bacterial pangenomes. By accounting for shared ancestry, Goldfinder aims to distinguish true co-selection from

spurious associations due to linkage or common descent.

We also enabled Goldfinder to integrate experimental phage-host range data, in order to identify bacterial genes that either promote or inhibit phage infection. This joint analysis can uncover candidate genes involved in phage attachment, defense, and counter-defense systems.

Finally, we highlight how convolutional neural networks can be used to identify the rate of gene transfer in bacterial populations and identify genes that have been acquired from a different lineage.

Together, these approaches provide a framework for understanding the genetic basis of phage-bacteria interactions and horizontal gene transfer and their role in shaping microbial pangenomes.



Coevolution of ploidy and sex

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POSTER SESSION 3 - P03.327, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Explanations for the evolution of sex often focus on the effects of recombination between selected loci, though the sexual cycle also comprises transitions between haploidy and diploidy. Prior modelling found that complete diploidy is favoured when deleterious mutations are partially recessive, since the full fitness effect of the mutations is masked. However, the reduced visibility of mutations to selection results in a higher mutation load, leading to haploidy when diploidy alleles are linked to the load. These models assumed that meiosis occurs

every generation, however many organisms have prolonged periods of haploidy or diploidy through mitotic reproduction. The overall rate of recombination therefore depends on how often meiosis occurs. We model the coevolution of sex and ploidy through two modifier loci that control the rate of sexual vs mitotic reproduction in each phase.

Populations evolved towards either complete haploidy or predominantly diploid life cycles, in which sex enables periodical purging of the genome.



Co-evolutionary dynamics of dietary breadth and body size in moths

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POSTER SESSION 1 - **P01.348**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Dietary breadth, or the range of food types consumed by an animal, plays a critical role in ecological and evolutionary dynamics. At the most fundamental level, it shapes how organisms interact with each other and with the physical environment to obtain food resources. In herbivorous insects, dietary breadth is closely linked to ecological niches, and the degree of dietary specialization has long been recognized as a key driver of speciation and phenotypic evolution. From a functional perspective, recent studies have highlighted relatively consistent positive correlations between dietary breadth and body size in herbivorous insects, particularly within Lepidoptera. Although relevant, such correlations alone offer limited insights into the co-evolutionary dynamics of dietary specialization and body size. To investigate these dynamics in greater detail, we reconstructed the evolutionary history of dietary breadth and body size in nearly all known northern European moths of the family Geometridae ($n = 372$).

Material and methods: Using continuous-time Markov models of discrete character evolution, models of continuous trait evolution that incorporate rate heterogeneity among branches, and statistical models derived from quantitative genetics, we estimated: (1) transition rates between different degrees of dietary breadth; (2) directionality patterns in the evolution of dietary breadth; and (3) whether directional changes in dietary breadth are associated with the evolvability of body size. We predicted that transitions between states of dietary breadth (monophagous, oligophagous, polyphagous) would not occur

with equal probability and that the evolvability of body size would be non-random with respect to changes in dietary specialization. Specifically, we hypothesized that the evolvability of body size would be negatively affected by instabilities in dietary breadth (i.e., elevated number of shifts in the states of dietary breadth), suggesting that the evolution of body size and dietary breadth proceeds in a relatively ordered manner.

Results: We found empirical support for most of our predictions. First, evolutionary transitions between more specialized states (monophagous and oligophagous) were considerably more frequent than transitions to or from the polyphagous state, although the recovered pattern still suggested a directional trend toward increasing dietary breadth. Second, the evolvability of body size was significantly associated with ancestor-descendant shifts in dietary specialization: body size evolution slowed down when shifts resulted in increased dietary breadth (i.e., descendants becoming more generalist than their ancestors). Third, we detected a weak but significant negative correlation between the cumulative amount of evolutionary changes in dietary breadth and body size.

Conclusions: Evolutionary transitions between pairs of dietary strategies are not random, nor is the evolution of body size independent of shifts in dietary breadth. Ancestor-descendant transitions toward more generalist feeding strategies were associated with reduced exploration of body size trait space, and instabilities in dietary breadth may have contributed to a reduction in body size throughout the evolutionary history of the species.



Communicating science with art – you don't need to be an artist!

Scott M¹

¹University Of Aberdeen

POSTER SESSION 3 - P03.336, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

As researchers, we understand the power of a good figure, it can make our work more accessible, memorable, and shareable. Visuals play a crucial role in science communication, helping to engage audiences both within and beyond academia. Science art takes this a step further, using creativity to translate complex ideas into compelling, visual narratives. And the best part? You don't need to be a trained artist to get started.

In this poster, I share my journey into science art—from self-taught beginnings to creating live illustrations of conference talks and

developing 'Allie the Amphipod', a cartoon mascot that helps explain my research. I've used these visuals in presentations, posters, and even as stickers that extend the reach of my work long after a conference ends.

I'll offer practical tips and tools for anyone interested in using science art to communicate their own research, no fancy equipment or art background required. Whether it's simple line drawings, illustrated methods, or mascots, science art can boost engagement on social media, enhance talks, and make your research stick in people's minds (and on their water bottles).



Comparison of genetic effective population size estimates in species across a large range of life-history strategies

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POSTER SESSION 3 - P03.283, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Population genetic diversity (GD) is essential for long-term adaptive potential of wild species and is shaped by life-history traits (LHTs) and microevolutionary processes. International conservation policy has endorsed the protection of GD with the adoption of a Headline Indicator in the 2022 Kunming-Montreal Global Biodiversity Framework: “the proportion of populations with an effective population size N_e above 500”. To shed light on the drivers of population N_e and improve GD indicator estimation from genetic data, we developed a categorization framework and estimated N_e for a selected set of 25 DNA datasets with rich metadata. The species represented with these datasets harbour contrasted LHTs strategies that relate to lifespan (axis 1), the spread of reproductive stages throughout lifespan (axis 2), and, additionally, to spatial genetic structure, dispersal capacity or population history (axis 3). For each species,

we compared N_e estimates obtained from two methods, based on the linkage disequilibrium in populations, or based on a Bayesian method including various genetic summary statistics as prior information. We also considered technical data features such as the type of molecular marker, and the number and quality of SNPs. Our results show a wide range of N_e estimates within some species (including populations with N_e below the 500 threshold and others above it), and also among species within the same categories of LHTs. We discuss challenges for obtaining reliable DNA-based N_e estimates for GD indicators in species with contrasted life-history traits variation, life cycle and evolutionary scenarios. By exploring these challenges, we aim to contribute to developing best practices for GD indicator estimation for nature managers.



Comparison of molecular diversity of spring fen macrozoobenthos from two geomorphological regions

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POSTER SESSION 2 - P02.031, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Recent studies on the molecular diversity of stream amphipods suggest that the Western Carpathians may have served as an important glacial refugium for freshwater fauna. This is supported by high variation of the local amphipods and presence of ancient and locally endemic lineages in comparison with the adjacent, more westerly located areas. If this scenario is true, a considerable molecular diversity of other aquatic taxa can also be expected in the Carpathian biogeographical region. In our project, we aim to quantify and characterise the molecular diversity of the benthic macroinvertebrate fauna of calcareous spring fens (a well-studied and therefore convenient model community) of the Western Carpathians and compare it to the diversity of the same type of communities in the neighbouring Bohemian Massif, a region with a different geomorphological history. Our hypotheses were that 1) the amount of molecular operational taxonomic units (MOTUs) per each spring fen would be significantly higher in the Western Carpathians than in the nearby Bohemian Massif and 2) this difference would be more pronounced for the fully aquatic, passively dispersing fauna (e.g. amphipods, molluscs) than for groups capable of flying and thus active long-range dispersal.

Materials and methods: We applied the community metabarcoding (cytochrome oxidase I Illumina sequencing) approach to quantify and characterize the molecular diversity of macrozoobenthos in a selected habitat type (21 calcareous spring fens in the Western Carpathians and 8 ecologically similar spring fens in the Bohemian Massif). The metabarcoding approach allowed for the assessment of diversity patterns (lineage

richness and composition) across a wide range of invertebrate taxonomic groups, from which selected taxa were selected for further in-depth analyses.

Results: The overall molecular diversity within the invertebrates was slightly, but not significantly, higher in the Western Carpathians. The expected significantly higher MOTU diversity was found only in some taxa, such as Amphipoda or Trichoptera, but was not as a general pattern. Overall, aquatic passive dispersers were not more diverse in the Western Carpathians, hence both our hypotheses were rejected by our data. We observed a considerable lack of reference sequences in public databases (Barcode of Life Database, GenBank) for a large part of the detected molecular operational taxonomic units (MOTUs), indicating that the studied regions are not yet sufficiently covered by barcoding efforts and/or suggesting that there may indeed be a considerable unrecognised diversity of macrozoobenthos. The raw patterns of MOTU diversity were however biased by substantial share of pseudogenes in some taxa (e.g., amphipods, leeches or tabanids), while in other macrozoobenthos groups, putative pseudogenes were absent or rare.

Conclusions: Our results suggest that the overall molecular diversity of freshwater macrozoobenthos in the Western Carpathians is not significantly higher than in the Bohemian Massif, and the expected pattern holds only for specific taxa. However, the data obtained showed some other interesting patterns, such as unusually high pseudogene occurrence in some groups, or high levels of undescribed cryptic diversity in the others.



Conservation genetics in ectomycorrhizal fungi: estimating the effective population size (N_e)

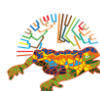
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POSTER SESSION 1 - **P01.350**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Effective population size (N_e) is a key parameter in population genetics and is increasingly used as a proxy for evolutionary potential in conservation genetics. However, challenges remain in the estimation of the effective population size in species where the definition of populations is complex. This is particularly the case for ectomycorrhizal (EM) fungi, a group of organisms of major importance for the functioning of terrestrial ecosystems, through the symbiosis they form with the majority of plants. EM fungi species display complex life cycles, with haploid and diploid stages, and both sexual and clonal reproduction. It is still unknown if methods developed for purely diploid and sexual populations can be reliably applied to estimate effective size in these organisms. We use genetic and genomic datasets from the literature to explore the effects of life history traits and genetic structure on the estimates of N_e in EM fungi. Datasets include basidiomycete species from diverse geographic ranges such as *Boletus edulis*,

Suillus brevipes, *Suillus luteus*, *Laccaria amethystina* and *Rhizopogon tomentosus*. Genetic methods to compute N_e include the linkage disequilibrium (LD) and sibship frequency (SF) methods, which are commonly used in conservation genetic studies. Our main findings show that the LD method is more sensitive than the SF method to the presence of clones in the data, i.e. the presence of replicated genotypes due to clonal reproduction. Thus, the SF method seems more appropriate for partially clonal species. We also show, as expected from the literature, that it is crucial to correctly delimit populations and estimate effective size at the within-population level, because mixing different gene pools tends to bias estimates of N_e , especially with the LD method. This work provides a comparative assessment of N_e estimates in different species of ectomycorrhizal fungi, providing insights for future research in conservation genetics for organisms with complex life cycles, especially partially clonal ones.



Conservation genetics of *Ambystoma dumerilii*, a narrow-endemic axolotl species critically endangered

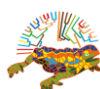
Lechuga Paredes P¹, Preziosi R¹, Puschendorf R¹, Garcia G², Williams L², Bamford P², Perez R³, Escalera L³, Dominguez O³

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POSTER SESSION 3 - P03.348, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The Ambystomatidae family is a group of amphibians, well known for its most recognized member: the axolotl. However, Mexico, houses another 15 native species; one of those native species is *Ambystoma dumerilii*, locally known as achoque. This amphibian is a narrow-endemic species from Lake Pátzcuaro, a high-altitude lake in central Mexico. The species is classified as Endangered by the IUCN and in Special Protection by the Mexican laws. The wild population of this species have suffered a dramatic population decline, due to over-fishing activities. For that reason, this PhD project is looking to assess the genetic diversity of some subpopulations to understand if such population decline has had an impact in the genetic diversity. The selected marker to assess the genetic diversity were microsatellites, as such tool allow to genotype each individual and include more salamander coming from wild and captive-bred populations during any stage of the project. The project seeks to genotype three subpopulations: salamanders from the wild, one captive bred

population kept in University of Michoacan, and one population kept in captivity inside the Dominican Monastery in Pátzcuaro town. Both captive-bred population might represent a genetic stock for future conservation actions. To accurately genotype achoque populations, a pool of 12 microsatellites coupled in three multiplexing groups have been tested and will be used to genotype most of the reproductive individuals. Such methodology will allow to estimate a more accurate population structure and genetic diversity, like the information that genomic tools, such as SNPs could provide, but with the advantage of having the genotyping of each individual. The microsatellites will allow to take informed conservation decisions and will allow to monitor the genetic diversity of the population in the near future. Additionally, these genetic markers could be applied to paternity tests in conservation actions for *Ambystoma dumerilii*, but this multiplexing groups will have the potential to be applied in conservation actions in other endangered Ambystomatids in Mexico.



Context-dependent mate preferences influence the stability of premating isolation in early population divergence

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POSTER SESSION 3 - **P03.320**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Understanding how premating isolation develops in the early stages of population divergence is a major challenge in ecology and evolution. Context-dependent mate preferences that change with environmental conditions could either promote or counteract the development of premating isolation. We quantified female mate preferences for resident and immigrant males within and between populations and across years in a colour polymorphic insect: the Common Bluetail Damselfly (*Ischnura elegans*). This species has three heritable female colour morphs, of which one is considered to be a “male mimic”. Closely located populations of this species differ in densities, female morph frequencies, species community composition and show modest genomic differentiation. We carried out mate preference experiments in years differing in environmental conditions and harshness, judged by female fecundity. We found that females expressed highly context-dependent premating isolation: in the benign year, females preferred immigrant males, whereas in the harsh year this pattern was

reversed and females instead preferred resident males. We also found that in the harsh year, field mating rates were low and females were more prone to copulate in our experimental mate choice trials, whereas the reverse pattern was found when field mating rates were high. Preference for immigrant males in the benign year was unlikely due to inbreeding avoidance, as nucleotide diversity in these populations is high. Instead, preferences for immigrant males when field mating rates are high are consistent with a modified version of the “chaseaway sexual selection” model, whereby females develop strong resistance against mating harassment from local males. Unexpectedly, we found no difference in between female morphs in these annual differences, suggesting that females respond similarly to changing environmental conditions regardless of their morph. Our results show that female mate-preferences are highly context-dependent and differ between years, with implications for the development and stability of premating isolation.



Cracking the transcriptomic hieroglyphics code: comparative transcriptomic analysis of respiratory organ evolution across arthropods

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POSTER SESSION 3 - P03.266, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Arthropods exhibit remarkable diversity in the morphology of their respiratory organs (from book lungs and gills to tracheal systems) despite all belonging to the same phylum. The evolution of respiratory structures was crucial for enabling them to transition from aquatic to terrestrial environments and face new challenges with oxygen availability and saturation - one of the most significant physiological shifts allowing them to conquer a new living environment. This raises a fundamental evolutionary question: do morphologically dissimilar respiratory organs share similar transcriptional underpinnings at a macroevolutionary level? To explore this, we analyzed gene expression across five tissues (including respiratory organs, brain, heart, leg muscle, and digestive system) in six arthropod species representing major evolutionary lineages (chelicerates, myriapods, crustaceans and insects), covering both terrestrial and marine species. With the goal to uncover whether shared or

distinct transcriptional architectures underlie the evolution of these morphologically diverse organs across species, we identified respiratory organ-specific “hub genes” (genes that are highly connected within a module and potentially play central roles coordinating tissue-specific functions) via Weighted Gene Co-expression Network Analysis (WGCNA), and explored their evolutionary origin through phylostratigraphic approaches. Our results revealed distinct clustering of gene expression profiles by tissue, with respiratory organ-specific transcriptomic profiles in each species showing functional convergence despite having a different evolutionary origin. This study offers a comprehensive understanding of how gene expression is organized within functionally similar yet morphologically different respiratory organs and how functional modules may have evolved across these species.



Crossing the biggest ocean in the world – the phylogeography of how plant genus *Wikstroemia* colonised the islands of the Pacific

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POSTER SESSION 1 - P01.140, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Wikstroemia, Endl., is a genus of bushes and small trees in the Thymelaeaceae family of flowering plants found throughout East, South, and Southeast Asia and on all major islands of the Pacific basin. They are a culturally significant plant to the native people of Polynesia, where the plants have long been used to make cordage, lei, fish toxins, medicine, and more. There are roughly 100 currently recognised species of the genus, a quarter of which have been reported in the Pacific. Many islands sport similar combinations of three to four species, Hawai'i being the notable exception where a dozen endemic species occurs.

Here, we investigate how the *Wikstroemia* genus has crossed the Pacific Ocean to successfully colonize and diversify on the many island habitats throughout Polynesia and the South Pacific? We present the first ever genus level phylogeny of the *Wikstroemia* genus, built on over 200 georeferenced herbarium and fresh material samples encompassing more than half the species and covering the genus's entire

range. We thus resolve longstanding inconsistencies that has haunted the taxonomy of the genus. Using this phylogeny as a backbone, we then reconstruct the biogeographic history of the genus: its origin in a common ancestor in Asia and subsequent dispersal across the Pacific region. Was each island colonised only once, or several times? Is the occurrence of several species across several islands the result of repeat colonisation events, or a taxonomic error caused by plasticity and parallel evolution? We discuss these questions as well as the “logistics” of cross-ocean dispersal and the challenge of defining species across vast geographic distances. We put specific emphasis on the colonisation of the Hawaiian archipelago – the most geographically isolated island group in the world, and a notable instance of rapid species radiation. We also discuss future prospects, such as why the *Wikstroemia* species present in Hawaii seem to have kicked the evolution of novel sexual systems in the otherwise hermaphrodite genus into overdrive?



Cytogenetic analysis of satellite content of five species of arvicolid rodents (Arvicolinae, Rodentia)

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POSTER SESSION 1 - P01.262, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Satellite DNA (satDNA) motifs play an important role in genome organization, centromere formation and chromosome segregation, protection of chromosome ends, gene regulation and speciation. satDNA motifs are among the most rapidly evolved parts of vertebrate genomes, resulting to significant differences in chromosome topology, copy variation and sequence similarity even among closely related species. satDNA is predominantly located inside and nearby heterochromatic regions, but extensive accumulations can be detected even in euchromatic regions. The arvicolid voles (Arvicolinae, Rodentia) are a group of mammals with a very high rate of karyotype evolution (eg. chromosome number and morphology) and extensive variability in the presence of heterochromatin (e.g. the emergence of the giant sex chromosomes). Therefore, we consider the arvicolid voles as a unique system to explore the evolution of the

satellitome. We conducted Illumina sequencing of genomic DNA at five arvicolid rodents (*Lagurus lagurus*, *Myodes glareolus*, *Microtus cabreræ*, *Microtus fortis*, *Microtus thomasi*), covering phylogenetically distant clades. We identified 29 different types of satDNA motifs by RepeatExplorer, we estimate abundance and sequence divergence in RepeatMasker, and subsequently, we uncover their topology on the karyotype by in situ hybridization experiments. Our results show that the majority of the satDNA motifs accumulate in centromeric and telomeric regions, as well as in the heterochromatic blocks of the sex chromosomes. In addition, we revealed a species-specific expansion of several satDNA motifs, as well as an amplification of satDNA specifically in sex chromosomes of certain species. Our study contributes to a deeper understanding of the evolutionary mechanisms underlying karyotype diversification in arvicolid voles.



Demographic history inferred from an inversion-rich spruce bark beetle genome

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POSTER SESSION 1 - P01.225, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The demographic history of species inferred from whole-genome data provides quantitative insights into key biological parameters such as population size changes and divergence times. Reliable estimates often require data that have not been affected by selection. However, extensive research indicates that many species harbour multiple polymorphic chromosomal inversions, which often evolve under different selective pressures. Consequently, inversions can influence genome-wide patterns of variation and subsequent evolutionary inferences. In this study, we used genome-wide data from over 300 spruce bark beetle (*Ips typographus*) individuals from 23 populations across Europe to reconstruct the demographic history of the species and to investigate the impact of complex polymorphic inversion landscape, covering approximately 28% of the beetle genome, on demographic inference. We used two complementary methods, Pairwise Sequential Markovian Coalescent (PSMC) and Site Frequency

Spectrum (SFS)-based modelling, and revealed a Late Pleistocene divergence (~79 kya) between populations from the southern and northern parts of the species' European range, and a long-term effective population size of ~250,000. The southern group underwent significant population expansion after divergence, whereas the northern group expanded during the Holocene. Recent population size estimates suggest that the southern group is twice as large as the northern group. Ignoring the presence of chromosomal inversions did not significantly affect the model selection procedure and resulted in relatively small biases in the estimated demographic parameters. This study provides information on the historical population dynamics of the spruce bark beetle and improves our understanding of the influence of complex genomic architecture on inference of evolutionary history.



Development of a Thermophilic PURE System for Cell-Free Paleobiology: Exploring Protein Evolution at the Origin of Life

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POSTER SESSION 3 - **P03.253**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The last universal common ancestor of life is thought to have thrived in thermophilic environments, making high-temperature conditions essential for understanding protein function and evolution in early life forms. Here, we present the development of a novel thermophilic PURE (Protein synthesis Using Recombinant Elements) system that forms the foundation of an emerging field we call "Cell-Free Paleobiology." This thermophilic PURE system features: (1) a cell-free protein synthesis platform with enhanced thermal stability using ribosomes and translation factors purified from thermophilic bacteria such as *Geobacillus stearothermophilus*; (2) efficient expression of ancestral proteins inferred through computational reconstruction methods; and (3) an integrated platform for structural and functional characterization under elevated temperature conditions. The key advantage of this approach lies in its dual freedom—

ancestral sequence reconstruction (ASR) provides "sequence freedom" while the PURE system offers "environmental freedom," enabling high-throughput functional analysis of ancestral proteins. Additionally, the fully defined cell-free environment allows precise recreation of various ancient Earth conditions (ion concentrations, pH, temperature) and experimental verification of translation system evolution itself. In this poster, we will share our progress in system development and preliminary validation experiments, highlighting specific applications including the analysis of molecular evolution in translation factors (IF2, EF-Tu) and functional characterization of ancestral Nitrogenase and Rubisco that played crucial roles in early life. This research is expected to significantly advance our molecular-level understanding of origins of life.



Discovering animal diversity through phylogenetic placement of 18S metabarcoding data

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POSTER SESSION 3 - **P03.339**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The small subunit ribosomal RNA (SSU rRNA) –18S in eukaryotes– is a universally present gene across the Tree of Life that has been instrumental to solve the most ancient relationships. Traditionally, 18S sequences are used to identify species or taxa. As a result, it serves as a molecular identifier of biodiversity in environmental DNA studies. Multiple methodologies have been proposed to identify known and novel biodiversity from environmental fragments of the 18S. One of those approaches, phylogenetic placement, arranges metabarcoding sequences into a phylogenetic context using a set of known reference sequences, known as the backbone tree. Despite its significance, specialised 18S databases still contain errors, which hinders the assembly of reliable reference phylogenetic trees needed to identify novel environmental 18S sequences.

We curated the most comprehensive 18S dataset of non-bilaterian animals in the Protist Ribosomal Reference Database. Using reliably identified sequences, we generated the largest backbone tree for non-bilaterians, essential for the taxonomic identification of molecular diversity within this group. Through phylogenetic placement, we are currently using our recently assembled backbone tree to analyse the avalanche of metabarcoding data generated by recent global expeditions such as Tara Oceans. With this approach, we mainly aim to unravel new diversity among non-bilaterian animals and to identify diversity patterns for such organisms. We also used this opportunity to study the suitability of the most common 18S-based barcodes, targeting their V4 and V9 regions, to phylogenetic placement.



Distinct sex-specific patterns in hematological and biochemical aging of Macaca monkeys

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POSTER SESSION 3 - P03.261, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Nonhuman primates are widely used in preclinical biomedical research, with hematologic and biochemical values serving as key clinical pathology parameters for assessing health status. In this study, we collected and analyzed 3,149 samples from 573 cynomolgus macaques (452 females, 121 males) and 193 rhesus macaques (91 females, 102 males). The data were categorized into three age groups: 1–7, 8–13, and 14–21 years. The corresponding human data was obtained from the NHANES database and incorporated into the study. Regarding white blood cell composition, neutrophil percentages increased while lymphocyte percentages declined until the expected age of puberty, a pattern observed in both our macaque data and the human data. Significant sex-based differences were identified in creatinine, alkaline phosphatase, and total cholesterol levels (Cohen's $d > 0.5$). Notably, creatinine levels

also showed significant sex differences in human data (Cohen's $d > 0.5$). Interestingly, red blood cell-related parameters such as red blood cell count, hemoglobin, and hematocrit exhibited the most pronounced sex-based differences in human data (Cohen's $d > 1.5$) and displayed slightly different age-dependent patterns between humans and macaques (Spearman $R > 0.2$). When examining age-related changes in macaques (ages 3–18) using R package DEswan, we found that the number of significantly changing parameters peaked at two distinct ages around 5 and 15 years in female while in male, there were one less distinct peaks roughly around age 7 or 8 ($\alpha = 0.05$). This study establishes reference intervals for hematological and biochemical parameters in macaques, providing valuable insights into primate aging.



Divergent visual system adaptations in two sturgeon species revealed by single-nuclei analysis

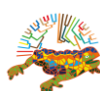
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*POSTER SESSION 1 - **P01.232**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM*

Sturgeons represent one of the basal lineages of Actinopterygii fishes. Despite their key phylogenetic position in early-diverging vertebrates, their visual systems remain poorly understood. Here, we present a results of single-nuclei RNA sequencing (snRNA-seq) of the retina in the sterlet sturgeon (*Acipenser ruthenus*) and starry sturgeon (*Acipenser stellatus*), two species with differentiated visual systems. The former has a common duplex retina containing both rods and cones, whereas the latter has lost rods during evolution. We profiled over 8000 retinal cells in both species and identified major neuronal subtypes, including cones (both species) and rods (sterlet). We identified multiple photoreceptor cone subtypes with distinct expression profiles - short-wavelength sensitive cones separated into two clusters (SWS1 and SWS2), and long-wavelength

sensitive LWS cluster. Rhodopsin (RH1) was highly expressed in the rod photoreceptor cell population. Sterlets likely have an ancestral state of retina organization with a “conventional” visual system enabling strong color discrimination and ability to see in dim-light conditions suggesting adaptation to visually rich environments and variable light conditions. In contrast to that, we confirmed that starry sturgeon does not express RH1, and lacks rods, resulting in lower ability to see in low light conditions. Interestingly, starry sturgeon also lost green opsins in cones, suggesting significant modification of vision. Given the lack of knowledge of starry sturgeon feeding behaviour and habitat preference these findings could have implications for the ecology of the species, yet we failed to connect this phenotype with any particular adaptation so far.



Diversity and adaptation in avian wing bone inner structure: insights from extant and extinct Strisores

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POSTER SESSION 1 - P01.368, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Birds' outstanding diversity stems from peculiar anatomical modifications, epitomized by the acquisition of wings. Studying wing bone morphology potentially elucidates how bird anatomy has been affected by intrinsic, e.g. phylogenetic, or ecological factors, e.g. flight styles. Although ecological factors have been shown to have limited impact on wing bone macroevolution, focusing on specific clades may reveal ecologically driven adaptations. The clade Strisores – e.g., nightjars/nighthawks, potoos and apodiforms (i.e. swifts + hummingbirds) – involves disparate and/or distinctive flight styles, representing a suitable context to identify ecologically driven wing bone characteristics. By relating wing bone traits to flight behaviors in extant species, we can potentially infer the paleoecology of fossil taxa.

Materials and methods: We studied humeral/ulnar inner osseous structure ('structure', hereafter), a level expected to be more ecologically driven compared to outer shape, across 11 extant Strisores species of hummingbirds, swifts, owl-nightjars, frogmouths, oilbirds, potoos, nighthawks and nightjars. We also studied two French Eocene–Oligocene taxa, i.e. Archaeotrogon, inferred as the sister taxon of nighthawks/nightjars, and Aegialornis, reconstructed as the sister taxon of oilbirds, hummingbirds and swifts. We quantified a wide set of structural traits from humeral/ulnar extremities - i.e. epiphyses - and shaft - i.e. diaphysis – and we compared them among the studied extant/extinct species. In phylomorphospaces, we assessed whether and which extant Strisores taxa are set apart according to their flight style, besides the structural traits mainly contributing to these trends. Moreover, we evaluated the position of Archaeotrogon and Aegialornis, to establish whether their wing structure mirrors more their closely related taxa or other extant Strisores.

Results: Despite the flight styles variety in Strisores, the only recurrent pattern across all the studied humeral/ulnar regions is the distinctive wing bone structure of hummingbirds, compared to all the other taxa. Hummingbirds' wing bones show traits which may be functionally related to loadings deriving from their outstanding hovering flight style, e.g. higher tortuosity and density of trabecular connections, more compact diaphysis. Interestingly, the chimney swift consistently tends to resemble hummingbirds for many structural traits. This pattern may be explained by this species being not as extremely aerial as other swifts. The studied extinct Strisores occasionally converge to hummingbirds, e.g. Archaeotrogon in the distal humerus, or swifts, e.g. Archaeotrogon in the proximal humerus, or tend to show a quite conserved structural configuration, i.e. Aegialornis in several anatomical regions.

Conclusions: Strisores' wing bone inner structure does not mirror the ecological and flight style variety of the clade, aligning with recent results on avian wing bone inner structure macroevolution. However, a tendency of hummingbirds to be set apart clearly arose. Hummingbirds being monophyletic, it is not possible to exclude that phylogenetic effects explain their distinctive morphology. However, their structural configuration may also relate to high loading likely deriving from the biomechanically demanding hovering flight style. The similarity to hummingbirds of one of the two studied swifts, also raises the possibility that hummingbirds' traits may be a plesiomorphic condition in apodiforms. While the wing bone structure of Aegialornis is quite consistent with its phylogenetic position, Archaeotrogon yielded some similarity with hummingbirds or swifts. Since Archaeotrogon is inferred as earlier diverging compared to apodiforms, its structural similarity to hummingbirds or swifts may suggest mosaic evolution and a quite disparate flight behavior.



DNA barcoding of passerine birds in Iran

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POSTER SESSION 1 - P01.192, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Exploring genetic diversity is essential for precise species delimitation, especially within taxonomically complex groups like passerine birds. Traditional morphological methods often fail to resolve species boundaries; however, DNA barcoding, particularly through the mitochondrial cytochrome c oxidase subunit I (COI) gene, provides a powerful complementary method for accurate species identification. This study establishes a comprehensive DNA barcode library for Iranian passerine birds, analyzing 546 COI sequences from 94 species across 23 families and 53 genera. We observed a pronounced barcode gap, with average intraspecific divergence at 0.41% and interspecific divergence at 18.6%. Notable intraspecific variation emerged in the Persian nuthatch (*Sitta tephronota*) and

the Lesser whitethroat (*Curruca curruca*), while the European goldfinch (*Carduelis carduelis*) and the grey-crowned goldfinch (*Carduelis caniceps*) showed limited genetic differentiation despite marked morphological distinctions. Phylogenetic analysis revealed significant east-west genetic splits in *C. curruca* and *S. tephronota*, reflecting Iran's geographic and zoogeographic boundaries. These findings demonstrate the effectiveness of DNA barcoding in elucidating biogeographic patterns, emphasizing Iran's key role as an ornithological crossroads for avian biodiversity. Moreover, our results suggest that much of the genetic variation in the COI gene arises from synonymous mutations, highlighting the role of purifying selection in shaping mtDNA diversity across species.



Do social bird species have more stable demographic histories?

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POSTER SESSION 1 - P01.344, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Cooperative breeding is a form of sociality whereby some individuals provide care to the offspring of relatives and occasionally to non-kin. It occurs across a large range of taxa, and is especially common in birds. It has been the focus of extensive research to understand its evolutionary drivers, highlighting important roles of various environmental conditions and life-history traits. However, the demographic consequences of cooperative breeding in particular, and sociality in general (e.g., affiliative behaviors), are less often studied.

Similarly to eusocial species (e.g., ants), albeit to a lesser extent, cooperative breeding species are often characterized by strong reproductive skews, with a few individuals monopolizing most of the reproduction. Reproductive skews could have consequences for the demographic history of a species, reducing the share of the population participating in reproduction, which should theoretically lead to a lower effective population size. Additionally, sociality is expected to buffer individuals against environmental fluctuations and ecological stressors, leading to more stable survival rates and allowing individuals to breed even during bouts of harsher ecological conditions, thus relaxing strong events of directional selection. The potential consequence is that populations of species with cooperative and social behaviors might

have more stable population dynamics and thus be more likely to persist through time, leading to more stable effective population sizes through macroevolutionary timescales. This reduction in effective population size and in its variability has been found in eusocial insects, as well as in comparative studies on snapping shrimps and spiders, however, the small number of species included limited their scope and conclusions.

Here, we use an unprecedented dataset of bird demographic histories in the last million years based on Pairwise Sequential Markovian Coalescent (PSMC) analysis of 636 whole genomes from 328 species spread across the avian phylogeny. Using various sociality measures (cooperative breeding, flock size, propensity to join mixed-species flocks) coupled with phylogenetic comparative methods, we aim to answer two questions. First, are cooperatively breeding bird species, which are characterized by strong reproductive skews, exhibiting markedly lower effective population sizes throughout their demographic histories? Second, are the demographic histories of social species more stable temporally, hence buffering them against environmental variation? This project provides invaluable insights on species' characteristics mediating populations and species genetic responses to changing environments.



Does the evolution for predatory aggression alter conspecific aggression? Insights from a selection experiment in bank voles

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POSTER SESSION 1 - P01.321, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Aggression, in its various forms, has been shaped by natural selection to facilitate survival and reproduction. Different types of aggression may have evolved to address different challenges, such as acquiring resources or defending against threats. The question of whether predatory and conspecific aggression are linked is often debated. Studying predatory behaviour and its relation to conspecific aggression may provide insights into aberrant human aggression, but animal models suitable for studying this in eco-evolutionary or biomedical contexts remain scarce. We used a unique experimental evolution model: lines of bank voles (*Clethrionomys* = *Myodes glareolus*) selected to hunt crickets (Predatory lines) and random-bred Control lines. The voles were subjected to selection for more than 30 generations, and to study intermale and maternal aggression, five males and five females were sampled from each of the four Predatory and four Control lines (total 80) from generation 36. Focal males and females underwent cricket hunting tests before and after mating to assess hunting aggression. On the 12th and 14th day after mating, the focal male was separated from its partner and placed on a neutral arena with a naïve opponent Control-line male. Post-parturition, on the 12th and 14th day, pups were separated from focal

female (mother), and an intruder Control-line male was introduced into this cage. The 20-minute aggression tests were videotaped and analysed for the latency, duration and frequency of aggressive behaviour such as boxing, wrestling, chasing and defensive responses like escaping, submissive postures and other activities. Focal voles from the Predatory lines showed greater prey capture success and aggression towards opponent males than voles from the Control lines. In intermale aggression test, Predatory males attacked the opponent male quickly, more frequently and for longer duration, while Control males exhibited higher vigilance by spending more time observing the opponent. Among Predatory males, boxing was the most frequent form of attack during the encounters. In the maternal aggression test, Predatory mothers had higher chasing and boxing behaviour than Control mothers, although the effect was not statistically significant. The Predatory voles explored the cage for longer time than Control voles, an indication of their proactive/bolder behaviour which supports conspecific aggression. Thus, selection for predatory aggression elevated conspecific aggression, and this effect was stronger for intermale aggression, suggesting a common genetic basis and the possibility of shared hormonal and neural mechanisms.



Does the social and sexual environment impact ageing through nutrient-sensing pathways?

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POSTER SESSION 3 - P03.285, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The sociosexual environment can determine lifespan; generally, reproduction is costly, but different types of social partners can be either positive or negative depending on context. Reproduction is usually more costly for females than males, and in some cases this expense is extreme enough to reverse the direction of lifespan differences between the sexes. Yet mechanisms underpinning sociosexual effects on lifespan in both sexes have proved surprisingly difficult to identify. Previous research has demonstrated that exposure to conspecifics can alter reproductive effort and gene expression in both males and females, including in the IIS/TOR nutrient-sensing pathway. This is a pathway that integrates a broad spectrum of environmental cues into downstream physiological responses, and so we anticipate that it will also play a central role in lifespan responses to sociality.

To investigate the potential role of nutrient sensing in mediating effects of the socio-sexual environment, we compare solitary male and female *Drosophila melanogaster* to those housed in mixed sex groups. We assess gene expression of nutrient sensing genes early in life to assess whether the different socio-sexual environment alters ageing trajectories through these pathways. We further explore this using a lifespan assay comparing wild-type flies in these same social groups, with a mutant line carrying a gene knockout of dFOXO (a key nutrient-sensing gene). If this gene plays a role in lifespan extension, we predict that we will see no difference in survival in this mutant line. Our results from these two studies will demonstrate whether sociality and nutrition influence lifespan using similar mechanisms.



Ecological and developmental mechanisms of floral diversification in selected South American angiosperms

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POSTER SESSION 1 - **P01.031**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Angiosperm flowers exhibit striking morphological diversity shaped by selective agents, such as pollinators with diverse forms and behaviors. However, evolution by pollinator-mediated selection is constrained by conserved patterns of flower development, entailing predictable growth-related changes in flower shape. Despite this constraint, pollinator-mediated selection can significantly change mature flower morphology. For instance, significant changes can be promoted through alterations in the timing of flower reproductive maturity in relation to growth, without altering the rate at which flower shape changes during growth. In addition, changes in flower morphology can result from alterations in the rate of shape changes during growth. Both of these mechanisms are examples of heterochrony.

Relatively recent orogenic and climatic events promoted a diversification of flower morphology in the South American flora, by driving changes in plant-pollinator interactions. Particularly, frequent shifts between bee and hummingbird pollination arose due to vicariance and the emergence of high-altitude environments during the Andean uplift. At shorter timescales, Pleistocene glaciations also impacted the pollination environment and generated complex scenarios of floral evolution. Pollination shifts between bees and hummingbirds significantly drove diversification in *Salvia* (Lamiaceae) and *Caiophora* (Loasaceae). Bee-pollinated *Salvia* species typically have shorter, open corolla tubes with bee landing platforms. Conversely, hummingbird-pollinated species evolved longer, tubular corollas matching hummingbird bills. Unlike *Salvia*, *Caiophora* lacks corolla tubes but possesses nectar scales (of androecial origin) that, together with the corolla, mediate pollination and have adapted to both pollinator types. Developmental mechanisms involved in morphological shifts related to pollinator change differ remarkably between *Salvia* and *Caiophora*. A comparison between two closely related *Salvia* species with contrasting corolla tube

lengths revealed that the longer tube in hummingbird-pollinated species resulted from prolonged organ growth in relation to reproductive maturity, largely due to prolonged anisotropic cell elongation. This suggests that *Salvia*'s pollination syndrome shifts during the Andean uplift involved heterochronic changes in growth trajectory extension, without changes to the relationship between size and shape during flower ontogeny. Conversely, in *Caiophora*, adaptation to hummingbird pollination involved changes in the rate of shape change relative to organ size. Hummingbird-pollinated species developed larger flowers than bee-pollinated species but retained juvenilized (paedomorphic) shapes, resembling those of bee-pollinated flower buds. This resulted, among other traits, in large and narrow corollas. Large flowers with a juvenilized shape in hummingbird-pollinated species arose likely from delayed epidermal cell differentiation relative to flower maturity.

Changes in the rate of flower shape development also facilitated intraspecific diversification in the oil-bee-pollinated South American genus *Calceolaria*. *C. polyrhiza*, an endemic species from Patagonia, experienced pollinator size transitions associated with recent Pleistocene glaciations. These shifts led to alterations in a key floral trait, the functional distance between floral rewards and fertile structures, matching different pollinator body sizes. Pollination by small bees repetitively involved a reduction in the rate of corolla shape development relative to corolla size, resulting in shorter functional distances. This indicates that, even over short periods such as since the Pleistocene glaciations, pollinator shifts can promote disruptions of the ontogenetic relationship between flower size and shape.

These studies of South American plant groups reveal how diverse ecological factors and developmental mechanisms together drove floral diversity, influenced by the geological and climatic history of the region.



Ecological disruption and evolutionary signals from transgene spread in a wild cotton metapopulation within its center of origin

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POSTER SESSION 3 - **P03.272**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The spread of genetically modified (GM) traits into wild relatives of crops raises biosafety concerns, particularly in centers of origin where ecological and evolutionary processes are closely tied to genetic diversity. In this study, we evaluated the presence and distribution of transgenes in wild cotton (*Gossypium hirsutum*) from two coastal dune sites within the Yucatán Peninsula Metapopulation (YPM), Mexico, using plant-associated ant communities as an indicator of their ecological effects. We found that more than 50% of the sampled individuals carried at least one transgene, with the copresence of both transgenes being common. In particular, we detected isolated expression of *cry2Ab*, a gene that does not exist as a single commercial event in Mexico, suggesting instability and segregation of stacked constructs in natural environments. The same pattern of transgene frequency and ant community

structure was observed at both sites: wild-type plants supported higher ant abundance and richness, while genotypes expressing *cry* genes were associated with lower ant abundance. These consistent trends highlight potential regional-scale ecological effects related to the introgression of transgenes into wild populations. In addition, we report the first record of *Cephalotes scutulatus* in the state of Yucatán, underscoring the ecological value of these habitats. Our findings emphasize the need to revise current biosafety frameworks to include evaluations of the stability, frequency, and community effects of transgenes, as well as how multiple transgenes interact, in wild populations. A more integrative approach is essential to understand the environmental impact of transgenes in natural populations and to support conservation strategies for native genetic resources.



Elastic solution for damage avoidance in genital lock-and-key evolution

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POSTER SESSION 1 - **P01.311**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Male genital morphology evolves rapidly in species with internal fertilization. According to the lock-and-key hypothesis, species-specific male genitalia ("keys") are adapted to fit corresponding female structures ("locks"), facilitating successful copulation with conspecifics. However, in areas where closely related species overlap, males may attempt to mate with heterospecific females, resulting in genital mismatches. Such mismatches can cause physical damage and impose direct fitness costs on both sexes. Therefore, selection may favor genital traits that reduce the costs of mismatched mating. While sexual selection within species has been well studied, little attention has been paid to selection on genital morphology in the context of heterospecific mating.

Materials and Methods: We investigated this issue in *Ohomopterus* ground beetles (*Carabus*, *Carabidae*), endemic to the Japanese archipelago, which exhibit species-specific lock-and-key genital systems. The male copulatory piece (CP) fits into the female vaginal appendix (VA), and mismatches between heterospecific CPs and VAs can cause damage. Notably, *Carabus maiyasanus* shows divergence in CP and VA length in regions where it contacts *C. iwawakianus*, consistent with reproductive character displacement (RCD) and supporting the lock-and-key hypothesis. We examined eight *C. maiyasanus* populations that differed in proximity to *C. iwawakianus*: four were geographically remote, two were parapatric, and two were isolated populations entirely within the range of *C. iwawakianus*. We measured geographic variation in CP shape and elasticity and conducted heterospecific mating

experiments using six of these populations to assess selection on CP length. We then analyzed how selection strength and direction varied across populations.

Results: Beyond previously reported RCD in CP length, we also found divergence in CP shape and elasticity. Isolated populations exhibited more elongated and more elastic CPs than other populations. At the individual level, elastic CPs were associated with more elongated shapes in one isolated population, while CPs were uniformly elastic in the other, suggesting adaptation to frequent heterospecific encounters.

Selection consistently favored longer CPs in the isolated populations. These longer, more elastic CPs reduced physical damage and shortened mating duration with heterospecific females, minimizing both physical and time-related costs. Interestingly, similar reductions in mating costs were also observed in one and two remote populations, respectively, indicating that cost-reducing genital variants may present before interspecific contact and substantial morphological divergence occur.

Conclusions: Although the lock-and-key hypothesis predates Darwin, empirical evidence has been limited. Our study provides the first direct evidence for selection on male genital morphology in heterospecific mating, consistent with RCD in lock-and-key systems. We demonstrate that longer, more elastic CPs reduce both damage and mating duration, potentially enabling males to better probe and disengage from heterospecific females. These findings reinvigorate support for a traditional but often dismissed hypothesis in genital evolution.



Emergence and evolution of angiosperm forests

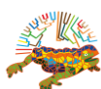
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POSTER SESSION 1 - P01.228, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The Angiosperm Terrestrial Revolution (ATR) refers to the diversification of angiosperms during the mid-Cretaceous to mid-Palaeogene (100–50 million years ago) that ultimately led to their dominance during the Cenozoic. This event led to a fundamental change in terrestrial ecosystems, with the emergence and dominance of angiosperm-dominated forests on Earth. A detailed examination of fossil wood records can provide valuable insights into the origin, evolution, and diversity of angiosperm forests. We have compiled data on angiosperm fossil wood worldwide based on an exhaustive review of the literature covering the Cretaceous and Paleogene. Our results show that, although exiguous, fossil records of angiosperm woody species existed from the Early Cretaceous, with a greater diversification and abundance towards the Late Cretaceous, and a dramatic increase in diversity by the end of the Cretaceous. We recorded nearly 150 genera of woody species by the end of the

Cretaceous (Campanian–Maastrichtian), suggesting high diversity in angiosperm forests at that time. Similarly, our analyses show that the diversification of woody angiosperms during the Cretaceous and Paleogene was marked by three key events that led to a decline in diversity at the family, genus, and morphospecies levels. These events include the K–Pg boundary, which had the most significant impact on diversification during the study interval, followed by the Paleocene–Eocene Thermal Maximum, and the Eocene–Oligocene Transition; the latter two events corresponding to significant climatic shifts. Cretaceous and Paleogene angiosperm forests were predominantly composed of eudicots, but their relative abundance in forests declined after the K–Pg extinction. Analysing these data enhance our knowledge of the diversification of angiosperms by uncovering evolutionary trends and identifying potential environmental factors that may have driven their diversification through time.



Environmental DNA illuminates evolutionary patterns in tardigrade communities across urban and elevational zones

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POSTER SESSION 2 - **P02.035**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Tardigrades, renowned for their ancient evolutionary history and exceptional cryptobiotic adaptations, are important models for investigating micrometazoan biodiversity and evolutionary processes over both ecological and evolutionary timescales. Their broad ecological tolerance and conserved morphology indicate a long history of survival through environmental changes and global climate fluctuations. Despite their evolutionary significance, the dynamics of tardigrade communities remain poorly understood, particularly with respect to how environmental zones—such as elevation and urbanisation—shape both micro- and macroevolutionary patterns. Environmental DNA (eDNA) metabarcoding has emerged as a promising method for revealing hidden diversity, including dormant and cryptic forms, which is essential for understanding these complex evolutionary processes.

Materials and Methods: In April 2024, we collected 73 moss samples from paired urban and rural sites situated at two distinct elevations in southern Poland. We recorded environmental variables, including temperature, humidity, and substrate type, to assess their potential influence on tardigrade community composition. eDNA was extracted using a homemade protocol, with COI and 18S rRNA markers amplified, followed by high-throughput sequencing on an Illumina MiSeq platform. A metabarcoding pipeline was employed to generate molecular operational taxonomic units (MOTUs), which were taxonomically identified using BLAST. We compared the taxonomic composition of the samples across environmental zones and cross-

validated findings with traditional microscopy techniques.

Results: Tardigrade sequences were successfully detected in most samples, revealing distinct differences in community composition between urban and rural zones, as well as across elevation levels. eDNA metabarcoding revealed a greater taxonomic richness compared to traditional microscopy, particularly in urban zones where eggs and other dormant forms were more easily detected. The homemade eDNA protocol proved to be both cost-effective and yielded results comparable to commercial kits, though gaps in reference databases limited the resolution of taxonomic assignments.

Conclusions: Our findings underscore the effectiveness of eDNA metabarcoding in uncovering hidden tardigrade diversity and elucidating community dynamics across environmental zones. From an evolutionary standpoint, the variation in tardigrade communities observed across urban and elevational zones may reflect not only ecological filtering and microevolutionary processes but also deeper macroevolutionary patterns, such as lineage divergence and historical biogeographic structuring. The detection of genetically distinct MOTUs consistent with cryptic speciation highlights the need for improved reference databases to better connect molecular data with evolutionary lineages. This study demonstrates the potential of eDNA as a powerful tool for studying the evolutionary processes and hidden biodiversity of cryptobiotic organisms, such as tardigrades, that are often overlooked in traditional ecological studies.



Environmental heterogeneity changes the genomic signature of adaptation to cadmium

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POSTER SESSION 3 - **P03.264**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Understanding the consequences of adaptation to human-altered environments has become increasingly important given the current anthropogenic actions. Heavy metals from agricultural and industrial activities can accumulate in the soil, be transferred to plants and propagate across all levels of ecosystems. Its short-term effects are well known across taxa. For example, at the molecular level, heavy metals can affect neurotransmission and cell homeostasis, leading to oxidative stress and cell death. However, we know very little about the long-term impact of heavy metals on organisms. Additionally, given that not all plants accumulate heavy metals, there is spatial heterogeneity within the environment, allowing for herbivores to either adapt to polluted environments or shift their ranges to non-polluted plants, affecting how they respond to the presence of heavy metals. Therefore, it is important to understand the long-term impact of heavy metal spatial heterogeneity on herbivore adaptation.

To fill this knowledge gap, we used experimental evolution of the two-spotted spider mite, *Tetranychus urticae*, maintained on tomato plants for 55 generations under three different selection regimes (i) plants without cadmium (control); (ii) plants with high cadmium concentrations (homogeneous environment); and (iii) plants with and plants without cadmium (heterogeneous environment). We used pooled whole-genome sequencing to estimate allele frequencies at SNPs across the genome in each regime. To detect SNPs under selection, we used a combination of statistical tests and simulations, by comparing allele frequencies in homogeneous or heterogeneous regimes relative to the control (no cadmium) regime.

After 55 generations of evolution, we detected significant changes in allele frequencies in SNPs along the genome for both cadmium-exposed regimes, consistent with the action of positive selection. Interestingly, we detected more SNPs under selection in the heterogeneous environment compared to the homogeneous environment. While we found that SNPs were spread along the genome for both selection regimes, the majority of SNPs detected on the heterogeneous regime were found in a small region on the first chromosome. Contrary to our expectation, we did not find signals of selection on metallothioneins, genes that play a crucial role in metal homeostasis and detoxification. Instead, we found changes in genes associated with neurotransmission, response to oxidative stress, transport channels and gustatory sensors in both cadmium-exposed regimes. We also found changes in specific genes for the different environments. On one hand, we found genes associated with lysosomal pathways only for the heterogeneous regime, potentially implicating changes in cadmium storage in cells. On the other hand, we found genes associated with the Golgi complex, namely with vesicle trafficking and secretion, only present for the homogeneous regime. Overall, our results suggest that, despite a common response to cadmium involving mechanisms to deal with oxidative stress and cadmium detection, spider mites evolving in spatially heterogeneous vs homogeneous environments adopted different strategies to deal with cadmium in their cells. Thus, our results highlight that environmental heterogeneity can modulate adaptation to environmental stressors.



Estimating Seagrass Meadow Persistence via Clone Aging

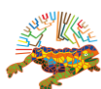
Winguth C¹, Endo Kokubun E¹, Yu L², Reusch T¹

¹Marine Evolution Ecology, GEOMAR Helmholtz Center of Ocean Research Kiel, ²CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences

POSTER SESSION 1 - P01.241, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Age distributions of clonal species remain elusive, limiting our knowledge on their demography. Through the aging of clones, we gain improved understanding of persistence of clonal populations, which is particularly important in the context of climate change. One widespread clonal species is eelgrass (*Zostera marina*). Among its many ecosystem services, eelgrass is home to many invertebrates and fishery nurseries, protects coasts from erosion, and stores blue carbon. Eelgrass can reproduce sexually or clonally, and thus genetic variation can reside at both levels of biological organization. Clones of eelgrass form via rhizome growth and branching with physical connections breaking apart after a few years, resulting in asexual populations of physically independent individuals (or ramets) belonging to the same genet

(sexually derived individual). Ramets of a genet accumulate somatic mutations through a process of somatic genetic drift that was recently identified, with the number of fixed somatic genetic differences being proportional to genet age. We applied a newly established somatic genetic clock to age eelgrass meadows in the western Baltic Sea at multiple locations. We used microsatellite markers to identify clones, which were then full genome resequenced. Using the accumulation of fixed somatic mutations and the mitotic mutation rate, we estimated the age of genets as a proxy for the minimal age of local seagrass meadows. These findings provide guidance for ecosystem management, as older seagrass meadows have more established ecosystem services, and shed light into eelgrass' current resilience despite clonality.



Evaluation of simulation-based supervised machine learning methods for inferring demographic parameters from genomic data

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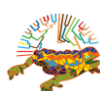
*POSTER SESSION 1 - **P01.197**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM*

The growing availability of high-throughput DNA sequence polymorphism data, alongside the development of numerous computational tools, has significantly advanced our understanding of the evolutionary and demographic history of populations. In this context, several demographic inference methods have been designed to harness the potential of these large-scale genomic datasets. Simulation-based approaches, such as Approximate Bayesian Computation (ABC), have proven particularly effective for analysing complex demographic models. However, fully exploiting the wealth of information embedded in massive genomic datasets remains a key challenge, as most inference methods rely only on partial summaries of the data. To address this, advanced computational techniques — particularly machine learning — offer promising solutions by enabling the integration of more comprehensive data features.

In this study, we demonstrate how simulation-based supervised machine

learning approaches applied to a wide array of summary statistics can effectively infer historical demographic parameters in connected populations. We compare the performances of three machine learning (ML) methods: a neural network (multilayer perceptron, MLP), and two ensemble techniques — random forest (RF) and the gradient boosting framework XGBoost (XGB). These methods were used to infer demographic parameters under both a standard isolation-with-migration model and a secondary contact model incorporating varying population sizes.

Our results show that the MLP outperformed both ensemble methods and relied on a broader combination of summary statistics, as revealed by permutation feature importance. Furthermore, we illustrate how SHAP (SHapley Additive exPlanations), a method from the field of explainable artificial intelligence, can be employed to elucidate the contribution of individual summary statistics within ML models.



Evolution of coordinated cooperation in kin groups

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POSTER SESSION 1 - **P01.194**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Sociality is one of the key aspects that characterizes humans. To achieve effective cooperation, one has to solve the coordination problem of cooperation, namely who contributes to public goods and who does not. This coordination problem is, however, vulnerable to liars who show the intention of cooperation but does not contribute any. Therefore, it remains a paradox how our behavioral tendency to engage in coordinated cooperation has evolved and been maintained in the population. We hypothesize that such coordinated cooperation was selected for in our ancestral population by kin selection and that it still persists in the present population

even after kin selection becomes weak or absent. To study this scenario, we have developed a new analytical machinery that enables us to study evolutionary game dynamics of multiple strategies played in kin groups. Our theoretical analysis reveals that, if the benefit function of cooperation has sufficient sigmoidal feature such that following through the coordinated cooperation is self-beneficial, cooperation remains prevalent even after kin interactions disappear. Our results provide a possible explanation of how cooperation is maintained in the modern human population.



Evolution of evolution: how do mechanisms of evolution change over time?

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POSTER SESSION 3 - P03.284, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Evolution of evolvability is an increase in the capacity of organisms to generate adaptations. However, during organisms' evolution, the strength and types of evolutionary factors can substantially change without any direct/immediate outcome for the capacity to adapt. Evolution of evolution can be defined as a change over time in the strength and types of classic evolutionary factors (mutation, selection, drift, and gene flow) affecting the population. In this study, I explore what the examples and mechanisms of "evolution of evolution".

Materials and methods: I perform a literature search to identify which traits of organisms can be part of the mechanisms leading to "evolution of evolution".

Results: The majority of identified traits that lead to the evolution of evolution can be classified into several categories: 1) genome structure (e.g., smaller genomes can have higher mutation rates and sometimes relaxed selection); 2) cell structure (presence of a nucleus could be a mechanism to prevent pervasive HGT, reducing its role in evolution); 3) morphology, physiology, and developmental plasticity

(high phenotypic plasticity reduces strength of selection); 4) life history traits (e.g., a longer life span can slow down generation turnover and reduce the strength of natural selection); 5) life cycle complexity (e.g., a haplo-diploid cycle can lead to the purge of deleterious recessive alleles, increasing the strength of selection). 6) External factors can also change evolutionary factors acting on the population (e.g., changes in habitat structure can alter gene flow dynamics).

Mechanistically, the evolution of evolution can be described as follows: the starting population of organisms at timepoint 1, under the influence of evolutionary factors, evolves into the population at timepoint 2. As a result, a population can evolve properties that alter the strength of evolutionary factors when the population evolves from timepoint 2 to timepoint 3 compared to from timepoint 1 to timepoint 2.

Conclusions: I identify at least 5 major groups of traits which can change the short-term evolutionary trajectories of populations and propose a mechanistic description the evolution of evolution.



Evolution of plasticity and evolvability in a model of bacterial surface exploration

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¹Ku Leuven

POSTER SESSION 3 - **P03.307**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

All organisms face environmental variation, to which they must be able to adjust their phenotype. Phenotypic change can result from genetic adaptation and plastic responses, and organisms can evolve to become more effective at either of these modes of adaptation. Bacteria are especially notable for their adaptive capacity. They have evolved extensive molecular machinery to accommodate fine-tuned plastic responses, but also mechanisms to evolve very rapidly. One such mechanism is phase variation, where hypermutable genes (contingency loci) alternate between two states through mutation, resulting in phenotypic variation in a population. Phase variation is often linked to immune-evasion in pathogenic bacteria, but the general conditions under which such an evolvability

mechanism evolves are unclear. Here, we present a basic simulation model in which bacteria evolve to explore a surface for nutrients. We focus on a major phenotypic switch observed in bacteria, between a free-living planktonic lifestyle and a surface-attached biofilm lifestyle. Bacteria need to be adhesive to exploit a nutrient patch, while motility is preferred for exploring. Adhesiveness is controlled by a gene regulatory network (GRN), which can evolve to become plastic and evolvable. We show how phase variation can evolve in this network as a mechanism to deal with environmental unpredictability. Additionally, we show how it becomes structurally integrated within the genetic architecture.



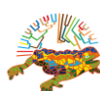
Evolutionarily distinct *Saccharomyces cerevisiae* lineages coexist within a single fruit habitat (marula fruits)

Tawanda Proceed M^{1,2}, Pontes A³, Boekhout T^{4,5}, Gibson B⁶

POSTER SESSION 2 - **P02.194**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Genome wide population studies have shown that *Saccharomyces cerevisiae* strains cluster into genetically distinct lineages largely defined by their ecological niche, geographic origin, and anthropogenic use. However, these foundational studies were based on a limited number of African isolates, leaving the continent's *Saccharomyces cerevisiae* diversity and its role in yeast evolution largely unexplored. In this study, a total of 10 *Saccharomyces cerevisiae* isolates, were isolated from marula fruits (*Sclerocarya birrea*) collected over two fruiting seasons from different locations in Botswana. To explore their evolutionary history, we conducted whole-genome sequencing of the *Saccharomyces cerevisiae* isolates and compared them to over 250 known domesticated lineages from across the globe. Phylo-genomic analysis revealed that the marula-associated strains represented five lineages, namely: African beer, Sake, Beer 2, a wild Chinese lineage, and a genetically admixed mosaic isolate. This diversity suggests that even within a single natural habitat, though rare, multiple

lineages with distinct evolutionary histories and phenotypic traits can coexist. The majority of the isolates were occupying basal positions within their respective lineages (e.g. African beer, Sake and the wild Chinese lineage), suggesting ancient divergence. The presence of an admixed isolate indicates potential natural gene flow between geographically and genetically distinct populations within this natural habitat. It is noteworthy that most marula-associated *Saccharomyces cerevisiae* isolates lacked common domestication signatures, such as aquaporin gene inactivation and starch degrading enzymes among others. The lack of strong domestication signals, along with the ancient phylogenetic placement of these strains, supports a scenario involving multiple, geographically distinct domestication events, possibly including an African origin. The marula fruit, a high sugar niche indigenous to tropical Botswana, appears to represent an underexplored reservoir of wild *Saccharomyces cerevisiae* diversity important in understanding its evolutionary history.



Evolutionary Biology Meets Artistic Research Across Scales

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POSTER SESSION 2 - P02.109, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Evolutionary biology is a science rooted in close observation, the use of coherent and structured logic and a deep appreciation for the object of study, qualities it shares with artistic research. In this work, we present three connected studies, guiding the reader from a cellular scale through organismal interactions to the broader forest ecosystem; each using indirect methods to reveal what is normally hidden. (i) The first study case is about *Tmesipteris oblancoolata* Copel., a fern from New Caledonia that holds the current Guinness World Record for the largest known eukaryotic genome. The amount of nuclear DNA is weighted by flow cytometry and the sheer size of the genome, poses questions that resonate both with evolution and philosophy. Through art we aim at connecting both perspectives and answer questions such as: why these ferns have so much DNA and what is the composition of their genome. (ii) The second project focuses on continuous monitoring of arthropods, recorded in the wild using high-resolution cameras and automated detection systems. Over thousands of hours of recording, we've

documented real-time interactions between inflorescences and their visitors. While this footage is rich in ecological information with evolutive implications, it also captures moments of love, violence, and coexistence among the species that visit the reproductive structures of one of the most specious botanical families, the Asteraceae. (iii) Finally, *Mil ulls m'observen* ("A thousand eyes watch me") uses night-vision camera traps in a woodland setting. This site-specific artistic installation provides insight into the life of the forest and its inhabitants, while simultaneously shifting the perspective to reveal the forest as an active observer, challenging the human monopoly on knowledge and positioning the ecosystem as a sentient, knowing entity. Together, these studies show how evolutionary questions can be explored through both scientific rigor and artistic sensitivity. By harnessing light in different ways and stepping back from direct observation, they uncover evolutionary narratives—from the vast scale of the genome size to the behaviour of arthropods and mammals—that often go unnoticed.



Evolutionary origin of vertebrate hematopoiesis: insights from lamprey development

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POSTER SESSION 1 - **P01.085**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

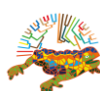
Background: The origin of vertebrates involved several crucial evolutionary innovations, including the development of a complex and highly regulated blood system. This system depends on hematopoiesis, a process responsible for generating all blood and immune system cells from hematopoietic stem cells (HSCs). These cells possess the ability to progressively differentiate into progenitors committed to specific lineages. In gnathostomes, transcription factors such as GATA1, RUNX1 and SCL/TAL1 have been identified as key regulators of a highly conserved gene regulatory network controlling different aspects of hematopoiesis. Despite the knowledge accumulated in gnathostomes, the origin of hematopoiesis and the cell types involved in vertebrates is not yet fully understood. In this regard, the Arctic lamprey (*Lethenteron camtschaticum*), a present-day cyclostome, the sister group of the jawed vertebrates, offers an exceptional opportunity to investigate the evolutionary origins of the hematopoietic program.

Materials and Methods: Lamprey and hagfish genomes and de novo transcriptomes were screened, followed by phylogenetic analyses, to find orthologous genes to important hematopoietic markers. The expression patterns of these were revealed by whole mount in situ hybridizations

lamprey development. Last, developmental single-cell map of lamprey development was performed by 3' scRNA-seq using 10X Genomics.

Results: Through genomic and phylogenetic analysis, we have identified multiple members of the gene families orthologous to key hematopoietic factors, including all members the GATA1/2/3, SCL/TAL1/LYL1, LMO1/2 families among others. We present here the complete expression panel of all these genes during lamprey development, including others previously identified such as RUNX genes. The expression patterns reveal specific spatiotemporal distributions allowing us to propose candidates with potential hematopoietic function. Last, we present a preliminary map of lamprey development at the single-cell resolution with a focus on the blood cell lineages, providing preliminary insights into the origin of the early hematopoietic process in vertebrates.

Conclusions: Our findings indicate that a regulatory program of hematopoiesis already existed in the common ancestor of all vertebrates, which evolved independently in the cyclostome and gnathostome lineages. This work constitutes a first step towards the functional characterization of embryonic hematopoiesis in lampreys.



Evolutionary Plasticity of Sex Determination: Multi-Omic Insights from a Rudimentary Hermaphroditic Sparid Teleost

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POSTER SESSION 1 - P01.142, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Teleost fishes exhibit remarkable diversity in reproductive strategies and sex determination mechanisms, offering a unique opportunity to study the evolutionary plasticity of sexual systems. Among them, sparid teleosts are particularly enigmatic, displaying a wide range of reproductive modes, including gonochorism, protandry, protogyny, and bidirectional sex change. Despite this diversity, the molecular and genetic bases underlying sex determination in this group remain poorly understood, and few clear cases of conserved sex-determining genes have been identified.

From an evolutionary perspective, the persistence of labile sex determination in sparids raises key questions about the genetic and endocrine mechanisms that guide the transition from undifferentiated gonads to stable sexual phenotypes. We investigate this in the Australasian snapper (*Chrysophrys auratus*), a marine sparid that exhibits rudimentary protogynous hermaphroditism, whereby individuals first develop ovarian tissue, which in males is subsequently replaced by testicular tissue before reaching sexual maturity. Using a multi-omic approach, we explore sex control by combining genome-wide scans for sex-linked loci with analyses of gene expression and hormonal signaling along the brain–pituitary–gonad (BPG) axis. For transcriptomic analysis, we sampled 104 fish during sexual differentiation across their maiden spawning season that were grouped according to histological sections, performing RNA sequencing of gonad, brain, and pituitary tissues to capture gene expression dynamics along the BPG axis at multiple timepoints and at high resolution.

Genome-wide association studies (GWAS) using SNP-chip data from 599 individuals revealed a polygenic basis of sex, with 1,589 SNPs significantly associated with sex after false discovery rate correction. However, no conclusive sex marker or master sex determining gene was found. Differential expression analysis (DESeq) identified 14,657 genes differentially expressed between males and females. Notably, the beta

subunit of follicle-stimulating hormone (Fsh)—a key hormone in the BPG axis—was downregulated in females. Quantitative Endocrine Interaction Network Estimation (QEINE) further suggested that Fsh has a significant role in inter-tissue endocrine signaling in males but not females, supporting a male-specific regulatory function for Fsh. Moreover, weighted gene co-expression network analysis (WGCNA) of gonadal RNA from 20 juvenile fish identified several gene modules associated with early male development. The most significantly male-biased module included the Fsh receptor gene and was enriched for genes involved in signaling pathways, including those related to sex steroid synthesis—processes known to play crucial roles in sex determination in other teleosts. By integrating histological phenotype data with genomic, transcriptomic, and hormonal data, we make significant progress in understanding how sex is determined in a sparid teleost, with implications that may extend to other teleosts and vertebrates with labile sexual systems. Our findings highlight major knowledge gaps in sex determination, suggesting a polygenic basis due to multiple sex-associated loci and apparent lack of a master sex determining gene. Furthermore, an environmental component of sex determination may exist, as supported by inconsistent snapper sex-ratios in aquaria, and the existence of environmental sex determination in other sparids. Given the role of Fsh in inducing sex change in some protogynous hermaphrodites, the prominent Fsh signaling observed here may reflect a conserved endocrine mechanism capable of triggering sex inversion—even in a functionally gonochoristic, rudimentary hermaphrodite with an apparently polygenic basis for sex determination. Ultimately, this study positions snapper at a transitional point in the evolution of their sex determination mechanism, possibly representing an evolutionary midpoint between environmentally controlled hermaphroditism and genetically controlled gonochorism.



Expansive pangenomes in Agaricomycetes

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POSTER SESSION 3 - **P03.321**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Trichaptum abietinum (Dicks.) Ryvarden (Hymenochaetales) is a pioneer wood rot mushroom

common across the Northern Hemisphere, usually growing on conifer wood. The species has emerged in recent years as a model for mushroom

genomics, and extensive genomic resources are available. Here, we have analyzed patterns of gene presence-absence variation across a dataset

covering *T. abietinum* known intraspecific geographical and genetic diversity. Our results suggest that *Trichaptum abietinum* has a large proportion

of its genome under Presence-Absence variation, over 40% of all genes for any particular strain. This pattern seem to be common across Agaricomycetes, but not across other groups of Fungi.

Core and accessory genes have distinct properties. Core genes also tend to cluster

towards chromosome centers, while accessory genes are more

abundant at the extremes. Core genes tend to be longer, have more introns and appear closer to other genes. Distribution of Accessory genes across

the species depends on genetic distance, but this variable alone can only explain a fraction of the observed pattern.

Unlike most other groups of fungi that tend to be haploid, Agaricomycetes spend most of their life cycle as a dikaryotic mycelium. Cell division in this mycelium is typically tightly regulated, which would be unnecessary were this a simple diploid. We hypothesize that extensive PAV has the potential of causing interference during mitotic recombination events, thus promoting genomic instability. This is then prevented by keeping the two nuclei separated until meiosis. This life cycle would allow these fungi to access a much larger gene pool than it would be possible for any single haploid genet.



Experimental evidence for cryptic female choice in the plant species *Brassica rapa*

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¹ISEM, University Montpellier, ²ISEM, University Montpellier, CNRS

POSTER SESSION 3 - **P03.304**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Sexual selection is known as a potent force shaping the evolution of diverse reproductive strategies in the animal kingdom. Sex-specific sexual selection typically arises from anisogamy, where females produce fewer and larger gametes than males, resulting in male-male competition for access to the limiting female gametes. Sexual selection theory therefore predicts its universal action across all sexually-reproducing anisogamous organisms, including plants. While the idea that sexual selection also operates in plants is now largely accepted, given their anisogamous nature, fundamental assumptions of sexual selection theory remain untested in the plant kingdom. Here, we test the hypotheses of classical ‘Fisherian runaway’ models of female choice, which assume a female phenotypic trait that directionally biases paternity toward a male trait, allowing females to produce offspring more efficient at accessing sexual partners or their gametes. Accordingly, we experimentally tested whether pistil morphology elicits female preference processes, known as ‘cryptic female choice’, during the phase of plant reproduction where the female and male components of mating physically interact.

Materials & Methods: In the angiosperm species *Brassica rapa*, we experimentally set up pollen competition among multiple donors by manually pollinating recipient plants. We tested for directional cryptic

female choice by genotyping parents and offspring, conducting paternity analysis, and quantifying pollen and pistil trait for all parental individuals. Specifically, we estimated whether multiple combinations of pollen and pistil traits acted in interaction to drive variation in pollen donor fertilization success.

Results & Conclusion: Validating the fundamental assumptions of Fisherian runaway models, pollen with higher germination rates fertilized more ovules, and this fertilization advantage was reinforced by pistil with larger stigma surfaces. Our results therefore confirmed that, as shown in several animals, a simple variation in pistil morphology can enhance pollen competition and favor sexual partners exhibiting more competitive pollen traits, consistently with a directional cryptic female choice process. Furthermore, pollen with slower growth speed fertilized more ovules in shorter pistils, while faster-growing pollen was more successful in longer pistils, conforming to a multidirectional female choice with two distinct preference strategies depending on the female trait. Our study revealed that multiple female morphological traits operate as selective filters on sexual partners, involving a diversity of interactions between male and female components of mating, highlighting the complexity of sexual selection mechanisms in the post-pollination phase.



Exploration of gene presence/absence variations in *Oncorhynchus mykiss* and their differentiation between wild and selection population

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POSTER SESSION 3 - **P03.233**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Gene presence/absence variations (PAVs) have been considered as the important determinants of genome evolution and phenotypic diversity. However, studies on gene PAVs have been poorly documented, especially in fishes. In the present study, pan-genome of rainbow trout was constructed based on 268 whole-genome re-sequencing accessions (4.38 Tb data). It recovered additional 62Mb sequences and 1288 protein-coding genes. Then, 9,831(22.77%) gene PAVs were genotyped across the 268 individuals. PAV-based PCA analysis, together with phylogenetic topology and STRUCTURE, revealed the clear separation among the different wild and

selection populations. Additionally, PAV-based GWAS has identified 3 candidate PAVs significantly associated with artificial selection. Meanwhile, Fst analysis revealed the 35 PAVs with significant frequency differences between wild and selection populations in Canada, while 15 candidate PAVs were detected between the populations in America. Their biological functions have been reported to participate in the regulation of growth performance and stress response. The present study would deepen our understanding of widespread gene PAVs and facilitate the identification of key candidates that contribute to important traits.



Exploring plumage polymorphism in birds of prey – a systematic map

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POSTER SESSION 1 - P01.285, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: The occurrence of different color morphs unrelated to sex or age is common among birds of prey. This phenomenon has attracted considerable scientific interest, leading to the development of numerous hypotheses, most of which are related to the ecology and life history of predatory birds. It is believed that the maintenance of different plumage morphs results from multiple selective pressures, but also genetic effects. Although previous studies have provided valuable insights into this topic, they focused on a relatively small proportion of species. In this study, we present the current state of knowledge, existing biases, and gaps related to the topic of melanin-based plumage polymorphism in birds of prey.

Materials and Methods: In our systematic map, we categorize the most and least studied aspects of raptors' ecology in relation to plumage polymorphism. Our approach considered both inputs—such as environmental and genetic factors influencing the presence of specific morphs—and outputs—such as the consequences of these morphs on adaptation, behavior, and fitness. We conducted a literature search using Web of Science and Scopus databases. Out of 1,767 articles screened, 297 were selected for full-text analysis. Finally, 213 articles were included in the systematic map.

Results: Our results show that most of the included studies focused on Strigiformes (54% of all articles), followed by Accipitriformes (32.4%) and Falconiformes (12.5%). In total, 30 focal species were identified, but the majority of studies centered on two owl species: the barn owl (30.5%) and the tawny owl (17.8% of all articles). Considerable attention was also given to the Accipitridae family (30.5%), with the most studied species being the common buzzard (8.5%) and the black sparrowhawk (7%). Within the Falconidae family, the highest interest was shown for kestrels (4.7%) and Eleonora's falcon (3.76%). For the remaining 24 species, each accounted for no more than 3.5% of the total articles, including single papers (0.47% of all studies) for 17 species.

Conclusions: We conclude that a significant portion of the evidence originates from a small number of research groups, often based on long-term studies of the same populations. We recommend increased efforts in physiological and genetic research to uncover pleiotropic effects and shared pathways between melanogenesis and hormonal regulation systems. These approaches may offer new insights into the mechanisms underlying plumage polymorphism in raptors.



Exploring the inbreeding dynamics in a cooperatively breeding mongoose.

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POSTER SESSION 1 - P01.074, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Inbreeding depression is the reduction in fitness in offspring of parents with shared common ancestry. Because groups often consist of relatives, cooperative breeders run particular risk of inbreeding and are expected to evolve inbreeding avoidance. Previous work on meerkats (*Suricata suricatta*), a socially cooperative mongoose, found that 44% of individuals had non-zero pedigree-based estimates of inbreeding (FPED) and demonstrated early life inbreeding depression. However, FPED estimates 'expected' inbreeding, not accounting for recombination and Mendelian segregation and in wild pedigrees is often underestimated due to missing pedigree links. Genomic based coefficients from runs of homozygosity (FROH), measure the extent of homozygosity across the genome that capture the realised individual variation in inbreeding. Using the long-term study of Kalahari meerkats, we developed a new genome-wide imputed SNP dataset (~7.3 million biallelic SNPs; 3086 individuals) and combined it with 30 years of field data to re-evaluate rates of inbreeding and inbreeding depression in fitness-related traits. A preliminary assessment using the

current pedigree confirms that 75% of the population (with at least 3 grandparents) have a non-zero FPED (mean = 0.046, range = 0-0.33), and finds that 9% have an $F > 0.125$, equivalent to half-sib mating. Although $FROH > 1\text{Mb}$ and FPED strongly correlate ($r = 0.80$) as expected, 99% of individuals have a non-zero $FROH > 1\text{Mb}$ (mean = 0.12, range = 0.0044-0.36) and 34% of the population have $FROH > 1\text{Mb} > 0.125$, revealing more evidence for inbreeding. Using $FROH > 1\text{Mb}$, inbreeding depression was found in the early life traits pup emergence mass, pup skull length, and pup forearm length. Only emergence mass is consistent between studies, with an individual with average $FROH > 1\text{Mb}$ (0.12) seeing a reduction of 4.3% in pup emergence mass when compared to an individual where $FROH > 1\text{Mb} = 0$. This result is less severe than previous estimates calculated with FPED (10.9%). By encompassing the variation missed by pedigree-based methods, we identify a far broader distribution of inbreeding and different effects on fitness-related traits. Future study will investigate the relationship between inbreeding and sociality in more detail.



Extraordinary adaptations: Functional and evolutionary synergy of trait components can explain the existence of leaf masquerade

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POSTER SESSION 1 - P01.347, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

One of the most enduring mysteries in biology concerns the evolution of complex adaptations made up of interacting component traits. When these component traits lack an obvious adaptive function in isolation from one another, their origin requires either non-adaptive, intermediate evolutionary steps or their simultaneous, synergistic evolution. We tested these alternatives using the powerful but accessible example of leaf masquerade in katydids, where in some species, highly modified wings strikingly mimic vegetation to

avoid predator recognition. Combining a field predation experiment with a phylogenetic comparative analysis of wing morphology in 51 Neotropical katydid species, we show that colour and shape synergistically interact to enhance survival in the wild, and modifications in both traits evolved concurrently during diversification of this clade. Our findings identify the functionality of masquerade camouflage in the wild and highlight how synergy between individual traits fosters evolution of extraordinarily specialised adaptations.



Floral developmental constraints and pollinator dependency in soybean

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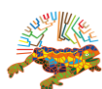
POSTER SESSION 3 - P03.346, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Cleistogamy, the production of self-pollinating flowers with a closed perianth at reproductive maturity, has evolved multiple times in angiosperms, and is also present in several crop plants including barley, rice, and soybean. In agriculture, cleistogamy is often viewed as a desirable trait because the closed, selfing, flowers maintain genetic homogeneity, ensure seed production even in the absence of pollinators, and protect floral reproductive structures against stressful environmental factors such as frost and pathogens.

Typically, individuals of cleistogamous species exhibit a mixture of closed (cleistogamous) and open, cross-pollinated (chasmogamous) flowers, with the proportion of open flowers increasing with temperature. Furthermore, cleistogamous species tend to exhibit premature pollen maturation within the anther locule, a phenomenon also speculated to affect the relative frequencies of cross- versus self-pollination. The frequent occurrence of plasticity in the production of the cleistogamous floral morph in response to temperature, and premature pollen production in species that evolve cleistogamy, suggests the presence of developmental constraints on these floral traits. It is important to further document and investigate the presence and correlation of these traits for important agricultural species to inform cultivar breeding efforts. Here, we present data documenting temperature-driven cleistogamy plasticity in

soybean, showing that the proportion of open, chasmogamous flowers increases with rising temperatures across six cultivars. This plasticity may help explain a well-documented latitudinal gradient of pollinator dependency in soybean, wherein plants at higher latitudes—and thus lower temperatures—exhibit reduced pollinator dependency, consistent with an increase in cleistogamy. Importantly, the soybean cultivars we investigated displayed significant variation in their degree of temperature-driven cleistogamy plasticity, indicating a genetic basis for the plastic response, and therefore the potential for this trait to be a selective breeding target for yield optimization. Additionally, we present evidence of premature pollen development (PPD) in soybean flowers. We show that PPD influences soybean's breeding system by limiting self-pollination, likely through the physical obstruction of pollen release, while facilitating pollinator-mediated cross-pollination. In this regard, we show that small wild insects are more effective in transferring pollen than honeybees.

These two findings underscore that soybean, even after intensive breeding and genetic modification, persists as a cleistogamous plant, exhibiting temperature-driven cleistogamy plasticity and PPD, characteristics shared with other cleistogamous species across the angiosperm phylogeny. Temperature-driven cleistogamy plasticity and PPD impact the plant breeding system, with potential consequences for soybean yield.



Fluctuating selection among years in a wild insect

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POSTER SESSION 1 - P01.361, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Temporal or spatial variation in selection has the potential to explain long-standing evolutionary problems such as evolutionary stasis and the maintenance of genetic variation. Long-term field studies of plants and wild vertebrates have provided some insights, but multigenerational measures of selection in wild invertebrates remain scarce. Short-lived ectothermic animals are likely to experience more pronounced environmental variation across generations than longer-lived and endothermic species. As a result, variation in selection may be particularly significant in these groups. Over 10 years, we have monitored an individually tagged population of wild crickets (*Gryllus campestris*) using a network of up to 133 day-night video cameras. The over a million hours of video that we watched allowed us to capture detailed information about naturally and sexually selected traits and life history

parameters. Over 10 discrete generations, the population size ranged from 51 to 546 adults. There were also substantial differences among years in the average values of traits including adult emergence date, body size, lifespan, and several behavioural traits. We combined measurements of these traits with individual fitness, measured as the number of adult offspring inferred from genetic-marker-based parentage assignments. This revealed substantial variation in selection gradients across years in several traits, with evidence that in one trait, adult emergence date, selection switched from positive to negative over the years. Our findings suggest that fluctuations in selection gradients are common but complete reversals in the direction of selection may not be very frequent



From Policy to Practice: Progress Towards Data-and Code-Sharing in Ecology and Evolution

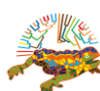
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POSTER SESSION 1 - P01.354, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

High-quality data and code are essential for ensuring the credibility of scientific findings. Data and code are crucial to facilitate reproducibility and providing them for peer review has several key benefits for both authors and reviewers such as error-checking and ensuring data and code adhere to best practices. However, data and, particularly, code, are rarely shared, even less so during peer review. Low sharing has been suggested to be driven by a lack of journal sharing policies as well as low clarity of existing policies. However, in ecology and evolution (E&E), we still do not know how

many journals have policies on data- and code-sharing for either peer review or upon manuscript acceptance. Here, I first present results on the links between clarity, strictness, and timing of data- and code-sharing policies across 275 journals in E&E. Then, I show the results from an analysis of initial policy compliance using submission data from two journals: *Proceedings of the Royal Society B* and *Ecology Letters*. Lastly, I will end with specific recommendations to help journals improve policy compliance and boost data- and code-sharing in E&E.



Gene flow between marine crab (*Liocarcinus depurator*) populations from the Atlantic-Mediterranean transition

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POSTER SESSION 1 - P01.357, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The marine crab *Liocarcinus depurator* is a decapod crustacean from the Polybiidae family which is very useful to study gene flow between populations. All larval phases are planktonic and can disperse over long distances due to marine currents. However, these currents can also produce eddies and gyres generating oceanographic fronts, thus reducing or impeding gene flow. In the Atlantic-Mediterranean transition the following oceanographic barriers may be observed: Gibraltar Strait (GS), Almeria-Oran Front (AOF) and Ibiza Channel (IC). The location and intensity of these fronts can change over time. For this reason, seven populations of *L. depurator* (Cadiz, West and East Alboran, Alacant, Valencia, Ebro Delta and North Catalonia) located along this region were analyzed in four consecutive years (2021-2024), and the results obtained were also compared with those from a previous temporal series (2014-2019). To study the molecular variation and connectivity between these populations, the COI (Cytochrome Oxidase subunit I) mitochondrial gene was used. Molecular variability parameters differed among populations and years. Also, using a haplotype network and a Neighbor Joining phylogenetic tree, all sequences obtained

could be classified in two clearly defined haplogroups: Atlantic (ATL) and Mediterranean (MED), being more abundant in Atlantic and Mediterranean waters, respectively. Using the GammaST genetic distance it was possible to detect significant differentiation between some populations. Especially interesting was the study of populations located at each side of a particular oceanographic front, since it allowed to deduce whether it presented a significant effect in a specific year. Thus, the effect of GS was significant only in 2021 (no data were available for 2022), AOF was significant in all studied years (2021, 2022 and 2023) and IC was not significant in any of the studied years. Furthermore, the correlation between genetic and physical distances was analyzed with a Mantel test. Despite the effect of some barriers was observed, this correlation was significant for 2022 and 2023. Finally, concerning genetic distances, a PCoA was computed for each year, and three regions were clearly differentiated: Cadiz Gulf, Alboran Sea and Levantine-Catalan region. These results could be very valuable to properly define MPA (Marine Protected Areas), and also for biodiversity conservation and sustainable fishing policies.



Genetic Basis of Immunity in *Drosophila melanogaster* Populations Selected for Improved Post-Infection Survivorship

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POSTER SESSION 3 - **P03.292**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Understanding how immunity evolves is essential for studying host-pathogen interactions. In *Drosophila melanogaster*, females often exhibit stronger immune responses than males, but the genetic and sex-specific mechanisms contributing to these differences remain unclear.

In this experiment, we investigated the genetic architecture and sex-specific immune responses in *D. melanogaster* populations evolved for enhanced survival post infection with *Enterococcus faecalis*, a Gram-positive pathogen.

Method: We generated F1 hybrids from four crosses: evolved male × evolved female (EE), evolved male × control female (EP), control male × evolved female (PE) and control male × control female (PP). We evaluated:

1. Post-infection survivorship,
2. PO activity at baseline (BI), 48 hours post-infection (HPI), and 96 HPI, and
3. Expression of key immune genes (dipterizin, drosomycin, dual oxidase (duox), and prophenoloxidase 1 (PPO1)) at the same time points.

Results: Survivorship was influenced by both genetic background and sex. EE flies had the highest survival, PP flies the lowest, and hybrids showed intermediate survival, indicating partial dominance of evolved alleles. Males consistently had lower survival than females, with the strongest sex difference seen in the PP group. However, dominance coefficients (Df and Dm) did not differ significantly, indicating no sex-specific dominance.

PO activity increased following infection in all groups but did not vary across crosses, suggesting a general inducible melanization

response independent of genetic background.

Immune gene expression varied by cross and time point, especially at 48 HPI:

- Dipterizin and drosomycin were strongly induced in EE, EP, and PE flies, particularly in females. PE males and PP flies showed minimal induction.
- Infection did not change the expression levels of Duox and the crosses did not differ in their baseline expression.
- PPO1 expression peaked in EE flies at 48 HPI, with little change in EP, PP females, or PE males.

Conclusions: Survivorship following infection depends on both genetic background and sex. Evolved alleles show partial dominance and enhance survival in both sexes. Although males had lower survival, no evidence of sex-specific dominance was detected. Similar survival in reciprocal hybrids indicates that immunity related alleles are probably on autosomes and we found no evidence of X-linkage. PO activity was induced similarly across genotypes, suggesting melanisation is not the key determinant of survival differences. Gene expression responses were variable and shaped by both genetic background and sex, with evolved flies showing stronger immune gene induction. These results suggest that selection has acted on both constitutive and inducible arms of the immune system. Overall, our findings highlight the complex interplay between sex and genotype in shaping immunity and suggest that evolutionary responses to infection involve multiple immune pathways, regulatory rewiring, and polygenic adaptation rather than changes in a single major immune effector.



Genome-wide assessment of three diverged lineages of common seadragon in the face of climate change

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POSTER SESSION 1 - **P01.315**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

West and East Australia are global hotspots for ocean warming, facing poleward contractions and extinctions of seaweeds and canopy-forming macroalgae. Several species disappeared from Western Australia as a result, and charismatic common seadragons (*Phyllopteryx taeniolatus*) have been decreasing at the outer margins of their range. Previous genetic work has elucidated species delimitation in this species and strong clines in genetic diversity, but the data was insufficient to distinguish historical from contemporary trends. For example, it is unknown whether gene flow is ongoing between three deeply diverged groups in West, Central and East Australia ($F_{ST} > 0.5$). In this study, we aimed to fill three important knowledge gaps: (i) define the influence of population demographic history on genomic diversity, (ii) identify the influence of gene flow on temporal population structure, and (iii) describe how these may influence adaptive potential in the face of climate change.

We performed whole-genome resequencing of 99 common seadragons. We analyzed population structure and gene flow in the ANGSD framework, which allows samples to have varying and sometimes low depth of sequencing coverage. To infer the population demographic history through changes in effective population size (N_e), we used pairwise sequentially Markovian coalescent (PSMC) plots. To identify functionally relevant SNPs in common seadragon genomes, genotypes within coding sequences were annotated with VEP and screened for homozygous High Impact (HI) and Loss of Function (LoH) mutations.

Our genomic data confirms that large genomic differentiation exists between West, Central and East Australia, despite their relative

geographic proximity. However, there is also evidence for admixture between these clusters, and recent gene flow seems to reach the northern parts of Tasmania, which is highly relevant for characterizing the connectivity of these populations. This points to long-term isolation with more recent evidence for secondary contact. A larger proportion of SNPs in admixed populations were inferred to be evolving under positive selection, and adaptive alleles under diversifying selection explained only a small proportion of genetic differences between the populations (<5%). Admixed individuals had, on average, shorter and fewer runs of homozygosity than non-admixed individuals and increased effective population size (N_e). Results also show that the maintained heterozygosity and N_e through time has been considerably lower in the East Australian cluster.

Our findings indicate that there has been recent gene flow between diverged common seadragon lineages, including gene flow across the Bass Strait. This study provides important and rare empirical evidence that small populations can maintain genetic diversity due to sporadic admixture between different genetic backgrounds and that admixed ancestry can promote the long-term stability of N_e and low genetic load (HI and LoH mutations). Signals of positive selection may suggest that the admixed variation may be contributing to the populations' adaptive potential. The lack of genetic variation is therefore unlikely to be the main stochastic factor causing the observed range contraction and population declines (reaching over 30% in parts of their distribution). Instead, the observed population declines are likely to result from extreme weather events, causing a rapid decline of suitable habitat.



Genomic and Transcriptomic Insights into Salinity Adaptation and Immune Trade-offs in the Spanish Toothcarp (*Aphanius iberus*, Valenciennes, 1846)

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POSTER SESSION 3 - **P03.287**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Salinity is a critical ecological factor in aquatic ecosystems, significantly influencing species adaptation and survival. The Spanish Toothcarp (*Aphanius iberus*, Valenciennes, 1846), a small killifish of the order Cyprinodontiformes, is endemic to the eastern Iberian Peninsula. This highly endangered species thrives across a wide range of salinity conditions, from freshwater springs and river mouths to brackish coastal lagoons and salt marshes. Its remarkable euryhaline capacity makes *A. iberus* an ideal model for studying genomic adaptations to osmotic stress and the physiological challenges faced by the species. Although *A. iberus* exhibits exceptional tolerance to salinity fluctuations, phylogenetic evidence suggests a saline-brackish ancestral origin for the genus. This implies that colonization of freshwater habitats could represent a more stressful condition, potentially triggering stronger immune activation (elevated gene expression) compared to populations from saline or brackish environments. An alternative hypothesis, linked to the non-migratory behavior of the Spanish Toothcarp (unlike diadromous species), posits that locally adapted genotypes may dominate in freshwater and saline populations, whereas brackish populations could face stronger trade-offs between osmoregulation and immune investment. To test these hypotheses, we examined genomic signatures of selection and transcriptomic differential expression,

focusing on gene families involved in salinity osmoregulation and immune response. We combined whole-genome resequencing and RNA-seq data from gills (the primary osmotic and immune barrier in fish) and gut tissue (critical for osmoregulatory water balance) across four populations spanning distinct salinity gradients. Integrating genomic (evolutionary) and transcriptomic (functional) perspectives allowed us to reconstruct the history underlying salinity tolerance in *A. iberus*. Our analyses identified candidate genes under positive selection and characterized their differential expression in response to salinity. We further investigated co-expression patterns between osmoregulatory and immune-related genes in both tissues, revealing potential functional trade-offs across osmotic niches. Key findings of this study include divergent selection patterns and differential expression in ion transport genes across salinity gradients in both gills and gut and suggest salinity-dependent modulation of immune components (e.g., interleukins, immunoglobulins, heat shock proteins), with distinct upregulation or downregulation patterns in freshwater, brackish, and saline environments. These results provide novel insights into how evolutionary history—whether ancestral legacy or recent adaptation—shapes physiological resilience and immune allocation in extremophile fishes.



Genomic basis of copy number variations and codon substitution provided the evidence for convergent evolution of salinity adaptation in teleosts

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POSTER SESSION 3 - **P03.238**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Salinity is a key ecological factor that influences aquatic habitats and regulates numerous physiological processes in fishes. Despite significant research, the overarching genetic and regulatory mechanisms enabling freshwater and seawater adaptation in teleost remain unclear and require further investigation. In the present study, a total of 26 different teleosts from 10 classification orders were selected to explore the evolutionary diversity in salinity adaptation between euryhaline and stenohaline species. Species tree suggested the discordance between phylogenetic classification and salinity adaptation ability, providing strong evidence for the independent lineages of salinity adaptation

in fishes. Following up the previous statistic method, more than 50 genes showed significant correlation between their copy number and salinity adaptation, including *slc12a10* and *otop2*. Meanwhile, the changes in gene copy number were largely agreed with their total expression abundance, which may regulate the gene function by the gene dosages effect. Additionally, BUSTED-PH method was performed to identify numerous genes underwent significant selection pressure between freshwater and seawater adaptation. Amino acid divergence could change the protein stability when confronted to different salinity environment.



Genomic basis of recurring phenotypic loss of bee scopae

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POSTER SESSION 3 - P03.328, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The loss of phenotypes represents a type of evolutionary innovation and is a widespread phenomenon. Like phenotypic gain, it can be adaptive and lead to new life histories. A particularly interesting case for evolutionary studies is the repeated loss of the same phenotype in independent lineages, because this allows for investigating the repeatability of evolution. Here we propose to study the genomic basis of repeated phenotypic loss using pollen collecting structures (scopae) in bees as example. These structures have facilitated the evolutionary success of many bee lineages as pollinators, but have been lost multiple times independently. This loss of morphological structures is accompanied by a transition to kleptoparasitism.

Being a female-specific trait (and an innovation itself), scopae are sexually

dimorphic, while the female-specific morphology is lost in kleptoparasitic bees.

Using a genome-wide comparative approach, we aim to reveal the genomic underpinnings that lead to the repeated evolution of that loss. We generated several dozens of novel genome assemblies to cover independent origins of kleptoparasitism and support our comparative approach with gene expression and network information relation to the (differential) development of these traits. Here we present a preliminary overview on multiple layers of comparative analyses, including transposable element and gene family dynamics, patterns of selection and gene loss. Our study reveals genomic underpinnings of repeated loss of bee scopae and illustrates the repeatability of evolution.



Genomic Insights into the Evolutionary History and Cryptic Diversity of Two-Toed Sloths (*Choloepus*) in Amazonia

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POSTER SESSION 3 - P03.330, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Amazonia has undergone significant geological and climatic changes over millions of years, shaping its rich biodiversity. However, the impact of key environmental events on the diversification of Amazonian species remains poorly understood. Two-toed sloths (*Choloepus* spp.) provide a unique model for studying this connection due to their wide distribution across Amazonia and restricted arboreal lifestyle, which makes them especially sensitive to shifts of the forest landscape. Previous studies have highlighted inconsistencies in phylogeny, karyotype, and morphology between the two *Choloepus* species, questioning the validity of current taxonomy.

Here, we present the first comprehensive genomic dataset representing different populations of *Choloepus*, using genome-wide nuclear and mitochondrial data to investigate their evolutionary history and phylogenetic relationships in relation to Amazonian biogeography. We find that *C. hoffmani* is paraphyletic, its southern

Amazonian population grouping with *C. didactylus*, supporting the need of taxonomic revision. Divergence time estimates based on nuclear loci suggest a more recent split between *C. hoffmani* and *C. didactylus* than previously inferred from mitochondrial DNA. The separation of South and East/Northeast Amazonian lineages coincides with Pleistocene climatic changes, which likely isolated populations via habitat fragmentation. Demographic reconstructions reveal population size oscillations during glacial periods, with the East Amazonian lineage exhibiting lower population size and genetic diversity, possibly due to the region's particular climatic instability and recent deforestation.

We emphasize the need for a taxonomic revision of *Choloepus*, as our findings reveal cryptic diversity and evolutionary divergence driven by Amazonian environmental changes. The distinct evolutionary lineages may require independent conservation assessments amidst the increasing threat related to habitat loss in Amazonia.



Genomic Insights of the Migratory Divide: Investigating Population Structure in the Red-necked Phalarope

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POSTER SESSION 3 - **P03.296**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Red-necked phalaropes (*Phalaropus lobatus*) are polyandrous shorebirds with a circumpolar breeding range. Recent research has shown that two geographically close breeding populations show highly distinct migration routes and wintering areas: Icelandic phalaropes migrate ca. 10,000 km over the sea to the tropical

eastern Pacific Ocean, whilst phalaropes breeding in Fennoscandia and Russia migrate ca. 6,000 km — largely over land — to the Arabian Sea. Here we test whether this migratory divide reflects population structure through PCA, STRUCTURE, and Fst-based analysis of whole-genome-sequencing data.



Genomic tools for identifying the origin of *Pinus pinaster* reproductive material

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POSTER SESSION 1 - P01.364, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: For organisms that disperse over long distances, such as forest trees, the ability to trace the origin of individuals is essential for conservation and sustainable management, as well as for research focusing on evolution and adaptation. However, genetic methods for origin identification have been underutilized due to methodological and practical constraints.

Material and Methods: We developed a genomic baseline for *Pinus pinaster* to facilitate genetic assignment at multiple levels. Using 10,185 SNP markers from 1,579 samples across 44 regions of provenance and 86 populations, we assessed the feasibility of identifying the gene pool, region of provenance, and population origin of individuals.

We first used two software packages to evaluate the reliability of our baseline dataset (i.e. reference data): RUBIAS, which specializes in genetic stock identification, and assignPOP, which uses machine-learning approaches for genetic assignment. We evaluated the accuracy and limitations of these methods in determining the origin of individuals. We then used the baseline to discriminate allochthonous vs autochthonous origin for 55 populations included in the Spanish National Forest Inventory.

Results: Our findings show that genetic assignment is highly reliable at the gene pool and region of provenance levels, provided a complete and well-structured reference dataset is available. However, accuracy declines at the population level, highlighting the challenge of fine-scale genetic discrimination. Incomplete baselines can lead to erroneous assignments, regardless of the sampling intensity. The commercial SNP array allows for the expansion of the baseline with additional samples, improving the accuracy of future genetic assignment, and is easy to use as a reference for new studies.

Conclusions: We provided a publicly available genomic baseline for *Pinus pinaster*, offering a valuable resource for conservation, forest management, and seed regulation. We then used genetic assignment methods together with this baseline to identify autochthonous populations. By enabling accurate provenance identification, our work supports adaptive management strategies for forest genetic resources in a changing climate. These genomic tools contribute to a better understanding of population structure and adaptation, ultimately informing conservation efforts and enhancing ecosystem resilience in an increasingly unpredictable environment.



Genomics and evolution of the expanding pine pathogen *Lecanosticta acicola*

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POSTER SESSION 1 - P01.116, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Lecanosticta acicola is the causal agent for brown spot needle blight that affects pine trees across the northern hemisphere. Recent studies have reported an on-going geographic expansion and an increased range of host species, both associated with climate change. Based on marker genes and microsatellite data, two distinct lineages have been identified that were introduced into Europe on two separate occasions. Despite their overall distinct geographic distribution, they have been found to coexist in regions of northern Spain and France. Here, we present the first genome-wide study of *Lecanosticta acicola*, including assembly of the reference genome and a population genomics analysis of 70 natural isolates from an infected forest from northern Spain. A comparative genomics study performed with a set of 28 closely related species revealed few differences in terms of number of secreted proteins or the presence of carbohydrate active enzymes (CAZY). Still, the presence of a secondary metabolism gene cluster was found conserved between the two pine pathogens

L. acicola and *Dothistroma septosporum*, pointing towards a possible role of this metabolite in pine infection. Comparison among different *L. acicola* isolates shows that most of the isolates belong to the southern lineage but show signs of introgression with northern lineage isolates. Admixture between northern and southern lineage strains is a finding without precedents worldwide that increases the previously expected evolutionary potential, production of genetic variation and the ability to adapt to new conditions of *L. acicola*, which could represent a threat to the already established management measures to control brown spot needle blight. The experimental measurement of a set of enzymatic activities in the different strains of *L. acicola* showed a clear divide between the phenotypes of the two populations with admixed strains having metabolic profiles closer to southern lineage strains. These phenotypic differences could explain the observation that strains from the northern lineage tend to be less virulent than members of the southern lineage.



Gestation length both shapes and is shaped by other life history traits in eutherian mammals.

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*POSTER SESSION 1 - **P01.307**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM*

The length of gestation in eutherian mammals, a key factor in their reproductive success, is closely linked to other life history traits, including body and brain mass. However, the direction of these relationships remains unclear. Here, we used an integrative analytical framework to evaluate the evolutionary relationships between gestation length and eight other traits across a dataset of 3,258 eutherian mammals, aiming to infer potential causal influences. Using a random forest machine learning algorithm alongside phylogenetic comparative methods, we identified variation in generation length and litter size as the primary predictors of gestation length. Additional traits, such as brain mass, were significant predictors only within specific mammalian orders. Applying phylogenetic path analysis, a structural equation modeling approach, we found that gestation

length directly negatively influences litter size across eutherian orders. In contrast, it directly positively influences brain mass in some orders and is itself positively influenced by brain mass in others. Furthermore, body mass directly positively influences gestation length only within the order Carnivora. These inferred relationships are supported by analyses of trait–trait coevolution, which reveal strong positive associations between gestation length and brain mass, strong negative associations with litter size, and moderate correlations with body mass. Our findings suggest that gestation length both shapes and is shaped by other key eutherian traits, largely in an order-dependent manner. This study offers a robust framework for exploring evolutionary relationships among suites of correlated and co-evolving traits.



Glowing and seeing red in deep-sea dragonfish (Stomiiformes)

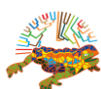
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POSTER SESSION 3 - **P03.334**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The ocean's twilight zone (200–1000 m depth) presents big contrast to terrestrial habitat. One of the main differences is the scarcity of light reaching 0.1% of ambient light; this can be compared to the cold, moonless sky in the Namib Desert. The second source of light is bioluminescence, produced by the oceanic invertebrates as well as vertebrates. Since ambient light is depleted towards blue-green wavelengths (480-570 nm) with increasing depth, the animals are also primarily adapted to perceive predominantly in this wavelength, and, accordingly, also to emit in the blue-green light spectrum. Dragonfishes *(Stomiidae)* are the one striking exception, being also able to operate in red to far-red wavelengths (around 650 nm) on top of the conventional blue-green. The bioluminescent chemistry is held in specialized glandular organs (photophores). The common scheme of luciferin functioning as a substrate for the enzyme luciferase, and this catalysis resulting in emission of light, is frequently more complicated and currently understood for only a slight portion of bioluminescent species. The red emission is obtained by a combination of different filters, reflective layers, and by transmission to a fluorescein (BRET) shifting the final emission to longer wavelengths. In this project, we are on the hunt for candidate gene(s) coding for

luciferase enzyme, or its cascade. By comparative transcriptomics, we were able to pinpoint a relatively narrow group of candidate genes. At first, we sampled 8 individuals from 4 species covering phylogeny of Stomiiformes (both red-glowing and blue-glowing species). By differential expression analysis of different types of photophores, we identified transcripts specific for photophores, and we complement those by the functional annotation. We believe that products of those candidate genes can be linked to bioluminescent machinery and might be involved in light emission. Our candidates would then serve for subsequent functional tests and AI predictions. In the future, we would like to use targeted proteomics to further evaluate the occurrence of proteins expressed by those genes in different types of photophores. We hope we will be able to identify gene(s) coding for stomiid luciferase enzyme. This would be a very important discovery, as one of the first vertebrate luciferases identified. The ability of some stomiids to operate in the red channel represents substantial evolutionary advantage, and we would like to see how those genes have evolved, including also other molecular context, such as how vision in red operates.



Heavy metal tolerance and bioaccumulation potential in fungi isolated from Kiruna Iron Ore mines

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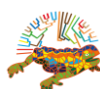
POSTER SESSION 1 - **P01.349**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Fungi are some of the most extremotolerant organisms on earth, and have evolved as key-players for extreme ecosystems. In order to broaden our understanding of adaptation to harsh environments, such as those highly influenced by anthropogenic activity, the critical first step is to characterize fungal species isolated from such environments.

Contamination by heavy metals is a major environmental problem brought about by many anthropogenic activities. The majority of heavy metals are toxic at low concentrations. The Kiruna mine, the largest underground iron ore mine in Europe, is a large industrial complex. Despite the high load of various heavy metals, several fungal strains have been isolated from environmental samples taken from the mine, six of which are *Fusarium* strains. Fungi have different means of coping with heavy metal stress, two of which include reduced bioaccumulation by extrusion of heavy metals, and increased melanin production binding to the metals.

In this study, we characterized the growth of six different *Fusarium* strains isolated from the Kiruna iron ore mine's sediments and four control *Fusarium* strains isolated from plants. Fungal strains have been grown at levels of 200 mg/kg, 600 mg/kg and 1000 mg/kg of three heavy metals, zinc, iron and copper.

We here describe the adaptation of fungi isolated from the mine to withstand higher levels of heavy metals. Mine fungi showed increased tolerance to each of the heavy metals compared to control fungi. Mine fungi furthermore grew better at low levels of heavy metals (200 mg/kg) compared to medium that was not supplemented with heavy metals. The growth of control fungi, on the other hand, rapidly decreased, even at medium supplemented with 200 mg/kg of heavy metals. At higher levels of heavy metals (up to 1000 mg/kg), mine fungi kept accumulating biomass while most control fungi ceased to grow entirely. Relative uptake of heavy metals into the mycelium of mine fungi was lower than in the control fungi, but the higher tolerance of mine fungi to withstand high levels of heavy metals leads to a higher overall bioaccumulation potential in these fungi compared to controls. Additionally, control fungi were darker in comparison to the mine fungi, suggesting higher levels of stress in control fungi. Together, our results provide a framework for understanding adaptation to heavy metal contaminated environments in fungi, and provide a basis for our ongoing studies on the influence of heavy metal tolerance on genome evolution.



Hidden Strength: Mutational robustness in changing environments

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POSTER SESSION 1 - P01.210, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Mutational robustness, the capacity of organisms to sustain their phenotype despite genetic mutations, may initially appear paradoxical. While it serves to mitigate deleterious mutations, it simultaneously reduces the number of beneficial mutations that can allow the population to adapt. However, it has been demonstrated that mutational robustness can shape the long-term evolutionary trajectories of biological systems in complex ways. For instance, mutational robustness mechanisms can facilitate the accumulation of cryptic genetic variation, which may be released when conditions change, thereby potentially enhancing the capacity of the population to adapt. The extent to which the rate and predictability of environmental change influence the evolution of mutational robustness mechanisms remains to be resolved. Here, we present the results of a computational evolutionary modelling study

that investigates the evolution of mutational robustness in populations under varying regimens of environmental change, as well as varying the cost of robustness. Our results show that mutational robustness emerges both if environments change only rarely, as mutations are not needed for adaptation under these scenarios, and if environments change very rapidly, when the buildup of cryptic variation is required to ensure the evolvability of the population under highly unpredictable circumstances. For intermediate environmental change scenarios, we observe that mutational robustness does not evolve. Overall, our results show that the dual effects of mutational robustness on mitigating harmful mutations and providing a reservoir of potentially adaptive variation has intricate consequences for its evolution across conditions of environmental change.



Holobiont plasticity and transcriptome stability in a gorgonian facing local anthropic pressures

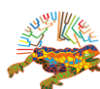
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POSTER SESSION 1 - P01.358, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Anthropogenic environmental changes impose new adaptive challenges for wild populations. This can question the evolutionary fate of these populations, especially regarding long-lived sessile organisms such as trees or corals. For these organisms, long-term genetic adaptation may be limited by extended generation time, and acclimatization should be particularly relevant in providing a short-term response. Changes in the composition of associated microbial communities may also play an important role in adaptation as posited by the holobiont theory. Here, we study the diversity of the holobiont of a Mediterranean gorgonian coral (Cnidaria, Octocorallia), and we test its plasticity in the context of local gradients of sea pollution near the city of Marseille (France). We performed a reciprocal transplant experiment between two sites, one near a major sewage outfall, and one further offshore. The experiment lasted six months. The environmental impact of pollution was estimated through the levels of inorganic contaminants in the gorgonian colonies, which showed contrasting levels of exposure between sites. Bacterial communities were analysed with 16S metabarcoding. An analysis of differential gene expression at the transcriptome level

was performed to study host plasticity. To get an integrated view of the diversity of the holobiont, we also investigated the genomic structure of the host to test for local adaptation, and to search for potential correlations between host genotypes and the composition of microbiome. The diversity of the microbiome before transplantation was characterised by higher diversity among colonies from the coastal site, close to the pollution sources, compared to the offshore site. Before transplant experiment, we identified a significant correlation between the microbiome composition based on Amplicon Sequence Variants (ASV) and the host genotype. At the end of the transplant experiment, we observed a change in the holobiont communities, but with substantial heterogeneity between colonies. The host transcriptome appeared less plastic with the main differences in transcriptome landscape corresponding to initial environment, and not to the final environment of the experimental transplantation. Our results point out how variations in holobiont compartments can be correlated or uncoupled. The results obtained are discussed in the light of the evolution of adaptive abilities in the context of short-distance heterogeneity in anthropised marine environments.



Host-Driven Divergence in the Fish Parasite *Ligula intestinalis*: Evidence of Parallel Evolution in Two Species Lineages

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POSTER SESSION 3 - **P03.291**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Parallel evolution, where independent lineages evolve similar traits in response to analogous selective pressures, offers insights into genomic adaptability and the predictability of evolution. Parasites, due to their intense host-specific interactions, present excellent models to study these phenomena. Our research focused on parallel adaptive evolution within the *Ligula intestinalis* cestode species complex, specifically comparing sympatric host-specific parasite populations from two lineages separated by more than 5 million years of independent evolution. The first lineage parasitises various cyprinid fish in Europe, while the other is endemic in Ethiopia and parasitises barbels. Utilizing double digest restriction-associated DNA sequencing (ddRAD-seq), we analyzed 95 parasite samples across both lineages, yielding 147,713 SNPs. The European parasites were sampled from *Rutilus rutilus* (roach), *Abramis brama* (bream), *Blicca bjoerkna* (white bream), and *Alburnus alburnus* (bleak), while the Ethiopian lineage

parasites were collected from *Enteromius humilis* and *Labeobarbus tsanensis* barbels. Our results revealed clear genetic divergence associated with particular host species within each lineage. We applied two independent selection scan approaches, OutFLANK and PCAdapt, to detect loci under divergent selection. In the European lineage, we identified 1,008 outlier loci, while 778 outliers were found in the Ethiopian lineage. Notably, we detected genomic regions consistently identified as outliers in both lineages, indicating parallel selection. Our preliminary functional annotation suggests these parallel-selected regions are involved in immune response modulation, reinforcing the hypothesis that immune evasion is a primary selective force shaping parasite evolution across distinct geographic lineages. Future investigations employing whole-genome sequencing are expected to uncover additional loci, thereby deepening our understanding of the genomic basis of parallel evolution and host specificity in parasites.



Host-shift changes patterns of morphological integration in a sex-specific manner: an experimental evolution approach in a seed beetle

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POSTER SESSION 1 - P01.122, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Modularity can be defined as the variation of integration within an organism, with some parts being more integrated and relatively independent of other parts, known as modules. The degree of integration of morphological traits can vary in phytophagous insects during host shift, when changes in food quality and chemical environment challenge them to adjust their developmental programme to newly imposed factors. Such stress can destabilise development and disrupt existing correlations between morphological traits. Since responses to environmental changes are often sex-specific, a change of host can also affect patterns of sexual dimorphism. Within a framework of experimental evolution, we conducted reciprocal transplant experiment with eight replicate populations of the seed beetle *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae, Bruchinae) adapted to common bean (*Phaseolus vulgaris* L., Fabaceae) as their ancestral host and to chickpea (*Cicer arietinum* L., Fabaceae) as a

suboptimal host. Using methods of geometric morphometrics we investigated the effects of short-term (single generation) and long-term (140 generations) host shifts on the changes in the patterns of morphological integration of two functional modules, thorax and abdomen in seed beetles of both sexes. Our results showed that the functional hypothesis of a two-module body was confirmed in females, suggesting that reproductive function plays the central role in forming patterns of modularity in response to environmental changes. Moreover, the degree of integration within two functional modules is higher in the ancestral host than in the suboptimal host, regardless of whether they evolved over one or more generations on the common bean. Our results suggest that host shifts in phytophagous insects can reshape patterns of covariation of morphological traits and potentially influence the evolutionary trajectories of populations in a sex-specific manner.



Hot stuff! Large brains buffer against heatwave induced cognitive decay

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POSTER SESSION 1 - P01.251, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Increasing temperatures negatively impacts behaviour with increased rates of aggression, and cognitive decay in humans. A growing body of literature reports negative effects of heatwaves on cognitive abilities that are important for fitness in wild animal populations. While a larger brain is known to generate enhanced cognitive abilities that can buffer against socio-ecological environmental changes. Yet, how a larger brain may buffer against heatwave induced adverse effects on cognitive abilities remains unknown. We examined detour problem solving and working memory during a seven-day long experimental heatwave in guppies artificially selected on relative brain size. Overall, detour problem solving was impaired among guppies during the experimental heatwave, while working

memory was unaffected. Importantly, large-brained guppies outperformed small-brained guppies in detour problem solving and working memory in both the heatwave and control temperature treatments. Specifically, when heatwaves caused cognitive impairment, guppies with larger brains exhibited cognitive performance levels comparable to those of smaller-brained guppies under normal temperature conditions. Our study suggests that small-brained individuals might have lower fitness when heatwaves impair the cognitive abilities required for survival and reproduction. Furthermore, the results open up the possibility that cognition driven brain size evolution may have been influenced by abiotic factors.



How do expression plasticity and pleiotropic gene function impact the evolution of gene regulation? A case study applying single cell multi-omics and environmental manipulation to embryos of divergently adapted fish

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POSTER SESSION 3 - **P03.295**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Gene regulatory dynamics integrate cellular context and environmental stimuli, and underlie the establishment of cellular identity and function, progression through development, and physiological processes. One important challenge is to understand how the sensitivity (or robustness) of gene expression to the environment impacts the evolution of gene regulation in natural populations. Since gene regulation happens within individual cells, and different cell types are required to respond differently to environmental change, there should be heterogeneity in environmental sensitivity between different cell populations. Additionally, genes can also have pleiotropic roles in several cell types and tissues, potentially imposing constraints on how they evolve. Here, we apply a multi-omic single cell assay of chromatin accessibility and gene expression to embryos of a marine and freshwater ecotype pair of threespined stickleback fish (*Gasterosteus aculeatus*), reared at different salinities. Threespined sticklebacks are a well-suited model to address interactions of plasticity and

evolution during adaptation - they experience a wide range of environmental variation and marine populations have repeatedly adapted to freshwater habitats. Using divergently adapted ecotypes combined with environmental manipulation enables us to evaluate both the effects of plasticity on expression divergence and the evolution of plasticity itself. By using a single cell approach, we can assess the relationship between levels of pleiotropy and expression divergence, and identify cell populations that are particularly plastic and/or targeted by evolution. Applying this to developing embryos allows us to assay entire individuals and cover a diversity of cell types. The multi-omic assay will provide insights into the regulatory underpinnings including cell type specific regulatory elements that respond to the environment and/or underlie adaptive divergence. Combined, we aim to improve our understanding of how plasticity and pleiotropic constraints shape gene regulatory evolution underlying rapid adaptation to different environments.



How subtle environmental differences influence the predictability of evolution?

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POSTER SESSION 3 - P03.288, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

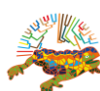
Understanding and predicting evolution is a central challenge in evolutionary biology. Despite several decades, we still lack a predictive framework of evolution, particularly due to the complex interplay between genetics and environment. One major limitation is the lack of a framework to quantify how environmental similarity influences the predictability of adaptation in evolving populations.

In this context, we design an experiment using chemically and metabolically similar environments to ask whether ‘subtle’ changes in environments can lead to predictable adaptive outcomes. Specifically, we study adaptation in environments that differ only in how two carbon sources, glucose and galactose, are structurally packaged and presented to microbial populations. These environments include two disaccharides (lactose and melibiose), and equimolar mixtures of glucose and galactose. We refer to these as ‘synonymous’ environments, as they contain

the two sugar monosaccharides, but differ in chemical linkage.

We evolved replicate populations of *Escherichia coli* and *Saccharomyces cerevisiae* for ~300 generations in these synonymous environments. Our results show non-correlated adaptive changes in populations evolved in ‘synonymous’ environments (in either organism), making predictability of evolution challenging. However, when fitness of the evolved populations were tested in a range of carbon environments, termed as non-synonymous environments, their pleiotropic responses were predictable based on the ancestral fitness in those environments.

Taken together, these results highlight the importance of environmental subtlety in shaping evolutionary trajectories and motivate the development of high-throughput evolution systems to systematically map pleiotropic effects across diverse ecological conditions.



Human-commensal songbirds and the genomics of convergent adaptation

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POSTER SESSION 3 - P03.306, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Author Mark Twain allegedly coined the famous conjecture that history does not repeat itself but often rhymes. Does this conjecture echo the evolutionary history of human-commensalism? The human-commensal house sparrow (*Passer domesticus*) could be the most abundant wild bird on Earth and likely became human-commensal in the wake of the agricultural revolution in Central Eurasia. From there the house sparrow spread across Central and Western Eurasia, capitalising on abundant cultivated grains. But in Eastern Eurasia, agriculture probably arose independently. And here, Twain's conjecture seems to hold true of human-commensal *Passer* sparrows: in parts of Eastern Eurasia, it is instead the tree sparrow (*Passer montanus*) which fills the human-commensal niche. So, in the independent wakes of the agricultural revolution, two closely related *Passer* species seem to have convergently evolved human-commensalism. Did the underlying genomic basis of adaptation to human-commensalism in *Passer* sparrows converge to repeat itself, too, or did it only rhyme?

We search for signatures of adaptation in the genomes of house and tree sparrows. European house sparrows were previously found to share a selective sweep for craniofacial development and digestive enzyme genes. To understand if all human-commensal *Passer* sparrows have a convergent genomic basis of adaptation, we

will compare geographically diverse house and tree sparrows to non-commensal ecotypes of each species. Through whole-genome resequencing and population genomics, we will elucidate the two species' population structure, history, and possible adaptations to human-commensalism. But already, we and prior work find that human-commensalism may not have had a single origin even within the house sparrow. The house sparrow species likely consists of multiple divergent but hybridising lineages. Therefore, we will also describe the origins and evolutionary history of the house sparrow writ large across its native range, from Northern Europe through Central Asia and to India. By studying house and tree sparrows, we can thus ask – both within and across species – to what extent the convergent evolution of human-commensalism has a convergent genomic basis in *Passer* sparrows.

Alongside ongoing biomechanical, morphological, physiological, and behavioural work in our group, this genomic research supports the use of *Passer* sparrows as an abundant and accessible model system for the study of adaptation. Besides, we hope to open a window on the natural history of these human-commensal songbirds with which different cultures have shared a close-knit history since the dawn of agriculture.



Identification of loci underlying local adaptation in European *Drosophila melanogaster* populations

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POSTER SESSION 3 - **P03.312**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Organisms are adapted to their local conditions and thus, populations differ across geographical distances. As environmental conditions often change gradually across these distances, gradients of genomic and phenotypic differentiation, or clines, have been observed. Despite major advances in revealing genotype-phenotype associations, the biological processes affected by genomic differences are still largely unknown. Thus, for many traits and model systems a thorough mechanistic understanding of genotype-phenotype associations for complex traits is lacking.

The European *Drosophila* Population Genomics Consortium (DrosEU) has recently established a resource of 160 isofemale lines for 9 natural *Drosophila melanogaster* populations across Europe. The lines had been distributed among multiple laboratories for an unprecedented phenotyping effort wherein 18 organismal traits including life history (e.g. viability, developmental time, fecundity, lifespan), morphology (e.g. thorax length, wing area, wing shape, pigmentation), physiology (e.g. dry weight, cold-shock and heat-shock mortality, chill-coma recovery time) and behavioural traits (circadian eclosion timing, circadian locomotor activity) were

quantified. Among these, candidate traits were identified that show signatures for local adaptation.

To link genomic variants to variation in organismic traits, we first sequenced the genomes of all 160 isofemale lines. We then compared genomic population diversity to previous pool-seq samples of the same populations. PCA analysis showed that most of the individual lines clustered by population. Almost 6 million SNPs were called among the 160 lines and genome-wide association studies (GWAS) will be performed for all previously measured organismic traits. To complement GWAS of organismic traits, we will study genome wide patterns of gene expression (RNAseq) and chromatin accessibility (ATACseq) in tissues with relevance for observed organismic phenotypes: the adult gut, the head, accessory glands and testes. The gene expression and chromatin accessibility data for the guts and heads will be obtained in the context of mating, thus also elucidating the population level differences between unmated and mated females. Eventually, the multi-omics data will be integrated to reveal high-confidence loci responsible for local adaptation.



Identifying candidate genes for extreme growth variation in response to nutrition in the water strider *Microvelia longipes*

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POSTER SESSION 3 - P03.265, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Morphological traits can respond differently to environmental cues. Among these, the exaggerated growth of plastic traits in response to nutrition are some of the most widespread examples in nature. However, the molecular mechanisms underlying this process remain poorly understood. In this study, we investigate the water strider *Microvelia longipes*, where males develop extremely elongated rear legs whose growth is highly plastic and influenced by nutritional condition. Nutrition alters the overall range of leg length variation producing shorter or longer legs in poor or rich diets respectively, while preserving the hyperallometric relationship. To explore the molecular basis of this plasticity, we performed comparative transcriptomic analyses on males from an inbred line raised under poor and rich nutritional conditions, focusing on

individuals at the extremes of the phenotypic range. Using non plastic traits as control, we identified differentially expressed genes potentially involved in this response, including the genes BMP11 and Ultrabithorax (Ubx) known to play a role in leg length variation in this species. Surprisingly, Ubx was overexpressed under poor nutrition, where leg growth is limited—suggesting a dose-dependent role of Ub in modulating growth under the influence of nutritional input. We further present recent functional data revealing interactions between nutritional cues and key developmental regulators. This would constitute first evidence for an interaction between nutrition and a Hox gene in modulating growth variation in an exaggerated trait.



Identifying the most stable endogenous controls for RT-qPCR analysis of genes of interest in larvae and adult male and female fruit flies (*Drosophila melanogaster*) exposed to different concentrations of cadmium chloride using available statistical tools

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POSTER SESSION 3 - **P03.347**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Heavy metal pollution has been one of the most pressing environmental issues associated with increased industrial activity in recent decades. Consequently, the exploitation and use of natural heavy metal deposits have led to their accumulation in the ecosystems. Cadmium, as a non-essential heavy metal, has severely detrimental effects on all living organisms inhabiting contaminated environments. Significant research efforts have been directed towards understanding how natural populations adapt to such stressors, including investigating the molecular basis of adaptation and tolerance to certain pollutants, such as gene expression analysis using RT-qPCR. To quantify the expression levels of any gene of interest, reference housekeeping genes with the most stable expression levels under the analysed conditions serve as endogenous controls. Since there is no single endogenous control that remains stable across all potential experimental scenarios, suitable reference genes must be selected for each research context. Despite the growing interest in this area, there is still a lack of information on suitable endogenous controls for studies examining cadmium exposure in *Drosophila melanogaster*. For that reason, this study aims to identify the most reliable reference genes for analysing the expression of small non-coding RNAs and coding genes in

response to cadmium exposure in fruit flies. To this end, fruit flies were reared on standard *Drosophila* medium as well as on medium containing four different concentrations of cadmium chloride (CdCl₂) – 5 µM, 10 µM, 25 µM, and 50 µM. To account for potential developmental and sex-specific differences in reference gene expression, both larvae and adult flies (male and female) from all five treatment groups were then collected for the gene expression analysis using quantitative PCR. The gene expression stability of ten housekeeping genes (TBP, GAPDH2, RpS20, Mnf/FoxK, α-tubulin, Rap2l, Cyp1, rpL32, actin, and EF1a) and two non-coding RNAs (5S rRNA and U6 snRNA) selected for this experiment will then be evaluated and compared under the imposed conditions using geNorm, NormFinder and BestKeeper, followed by the statistical analysis. Results obtained in this study will identify the most suitable single reference gene and, when recommended, the optimal combination of multiple reference genes for this and similar experimental settings. Furthermore, this study will determine if there is a significant difference in reference gene stability between larvae and adult male and female flies, further specifying the most reliable combination of endogenous controls for each developmental stage and sex, that is exposed to various concentrations of heavy metals.



Impact of geography, seasonality and experimental selection on *Chironomus riparius* recombination rates

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POSTER SESSION 1 - **P01.355**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Recombination is a key driver of genetic diversity, selection, and adaptation, yet its variation and dynamics remains understudied in non-model species. While most research has focused on spatial patterns in model organisms like *Drosophila melanogaster*, less is known about temporal dynamics of recombination, particularly in response to seasonal or multi-generational environmental changes. Experimental evolution offers a powerful framework to explore such dynamics. Here, we investigate recombination rate variation in *Chironomus riparius*, a multivoltine species of non-biting midge, across natural populations (geographic gradients and seasonal transitions) and under controlled experimental treatments (temperature regimes, microplastics, and cadmium exposure).

Materials and Methods: We retrieved Pool-seq data from ENA for five published studies examining various aspects of the evolutionary and molecular ecology of *C. riparius*. Data preprocessing involved read trimming and alignment to a newly available chromosome-level genome. Recombination rates were estimated using ReLERN, a neural network-based method. Genetic diversity and F_{ST} were calculated using PoPoolation1 and PoPoolation2. We assessed relationships between recombination rates and genetic diversity, GC content, and F_{ST} using Bayesian statistics, and further evaluated causality using structural equation modelling (SEM), based on the

premise that recombination rates and F_{ST} influence genetic diversity.

Results: In natural populations, recombination rates did not display a consistent latitudinal pattern, likely reflecting complex interactions between climate-driven selection and regional environmental heterogeneity. Seasonal variation was evident, with higher recombination rates observed after the summer compared to after the winter, possibly due to temperature-induced plasticity or seasonal bottlenecks. A notable cold snap event led to a sharp increase in recombination, suggesting a stress-induced adaptive response. In experimental populations, thermal regimes did not directly influence recombination rates, but adaptation to laboratory conditions had a significant effect. Environmental stressors triggered similar responses: microplastics exposure led to a genome-wide reduction in recombination, likely due to stress-induced DNA repair mechanisms prioritizing genomic stability, whereas cadmium exposure generally suppressed recombination. The SEM generally fit the data well across all datasets, confirming most hypothesized causal relationships, particularly between recombination rates, genetic diversity, and F_{ST} .

Conclusions: Our findings highlight recombination as a highly dynamic process shaped by environmental factors, selection pressures, and genetic background, emphasizing its crucial role in structuring genomic architecture under both natural and experimental conditions.



Impact of landscape structure on trait variation

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POSTER SESSION 1 - **P01.352**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Land use is one of the key drivers in the current biodiversity loss with agricultural intensification and climate change impacting natural habitats worldwide. Landscape structure has the potential to filter individuals for example through structural changes or changes in microclimate which could lead to spatial sorting of individuals by their traits. One key trait impacting individuals' ability to survive in changing landscapes is dispersal which is also a key trait for the gene flow between populations. Studies assessing among species variation in dispersal suggest that species with better dispersal abilities are more likely to persist under intensified land use. Less is known about land use impacts on within species dispersal ability and subsequent consequences on population genetic structure.

Materials and methods: In this study we collected individuals of three grassland butterfly species (*Maniola jurtina*, *Pieris napi*, *Thymelicus lineola*) from six populations across two different types of landscapes; habitats surrounded by agricultural fields and more natural or semi-natural meadow habitats. These species were selected to present commonly found butterflies in both types of landscapes with differing dispersal capabilities. We measured variation in dispersal (morphological and physiological proxies) and body size. Individual whole-genome sequencing was used to assess population structure between the landscape types and populations in all species.

Results: Intra-specific variation in flight metabolic rate (FMR) was evident in all species. However, only in *M. jurtina*, which is

considered as an intermediate disperser in comparison to high disperser *P. napi* and low disperser *T. lineola*, butterflies originating from agricultural landscapes had higher FMR compared with butterflies originating from more natural landscapes. *M. jurtina* butterflies from agricultural landscapes had also higher body mass than those from more natural landscapes. Assessing the genetic structure between populations will provide information whether the differences in FMR in *M. jurtina* have also led to genetic structure between landscape types. The genetic analysis can also confirm our assumptions on the dispersal capabilities of the different species; with no genetic structure between populations indicating high gene flow and dispersal between populations vs. population structure indicating lower gene flow and dispersal. The sequencing is completed, and the analysis of the genetic structure are underway.

Conclusions: This study provides insight on the impacts of land use on variation in life history traits. Our results suggest that land use, at least in some species, can impact within species trait variation with agricultural landscapes selecting for individuals with better dispersal ability. The genetic analysis will help to shed light whether landscape structure can lead to population structure between landscape types or whether it is simply explained by geographical distance and dispersal ability. Further studies would be required to understand the impacts of landscape structure on ecological processes, both between and within species.



Impacts of management on the genomics of perennial crops: a case study of two mezcal *Agave* species with different selection histories.

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POSTER SESSION 3 - With Aperitif, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Particular wild plants and plants in incipient proceeds of cultivation are managed in different ways and contexts in Mesoamerica. The direct extraction and different agricultural practices can modify populations' genetic composition in the *Agave* genus. Currently, many agaves used in mezcal (a traditional tequila like alcoholic drink) production are intensively propagated to meet the national and international demand for this beverage. Two agave species facing strong selective processes are *Agave karwinskii* and *Agave cupreata*. Both species are ecologically, economically, and culturally important regional resources in southern regions of Mexico and are now intensely exploited due to the current demand for mezcal. The prevailing reproduction method in most agave species, such as *A. karwinskii*, involves underground rhizomes, facilitating the establishment of commercial plantations. However, species like *A. cupreata* do not produce basal shoots or bulbils, depending entirely on seed production, making nursery establishment indispensable for mass plant production. What genomic patterns can we expect in a species with dual reproductive modes and a restricted distribution, like *A. karwinskii*, compared to an agave that reproduces exclusively sexually and has a wide distribution, i.e., *Agave cupreata*? To address this question, we analyzed the genomic relationships within and between populations under different degrees of management-- from wild and minimally managed to commercially plantations--, to understand in the distribution of their genomic variation and whether it may be lost due to agricultural management. Individuals from both species were collected across their natural distribution: 39 localities for *Agave karwinskii* and 26 for *Agave cupreata*. Their

genomic DNA was used to make double-digested RAD-seq libraries. Ipyrad was used for assembly and SNP calling, using a transcriptome of *Agave tequilana* as a reference. Filtering included read depth, allele frequency, missing data thresholds, Hardy-Weinberg equilibrium, and linkage disequilibrium. We analyzed 7,156 SNPs from 300 individuals of *A. karwinskii* collected in the Mexican states of Puebla and Oaxaca and 11,852 SNPs from 142 individuals of *A. cupreata* collected in the states of Guerrero and Michoacán, estimating genomic diversity statistics and genomic differentiation patterns. From a genomic perspective, establishing Evolutionary Significant Units (ESUs) and Management Units (MUs) are essential for the conservation of each species. Our results indicate that in *A. karwinskii*, the populations from Puebla and Oaxaca should be considered separate ESUs; within each, MUs should account for the two reproductive strategies present (sexual and clonal). For *A. cupreata*, we propose two MUs, one corresponding to Guerrero and another to Michoacán. Although our results show an overall good genomic health for both species, we identified some populations apparently at risk. Current extractive management practices have overlooked the traditional selection method that once contributed to keep the genomic diversity, and if the management practices continue unchanged, we expect a steady decline of wild populations. Therefore, urgent management practices for both agaves should be implemented, including the establishment of nurseries, local germplasm banks and reforestation programs aimed at restoring pollination corridors and connectivity among populations.



Individual heterogeneity in bovine tuberculosis (bTB) disease dynamics in European badgers: genetics, senescence and social networks

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POSTER SESSION 3 - P03.302, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Spillover of infectious diseases from wildlife to humans and livestock poses one of the most pressing global threats to public health and livelihoods. Despite advances in understanding of disease processes in humans and domestic animals, the eco-evolutionary dynamics of infectious disease in wildlife remains poorly understood. This knowledge gap hampers One Health approaches to disease management and outbreak prevention, as well as our fundamental understanding of the eco-evolutionary processes that shape host-pathogen interactions in natural systems.

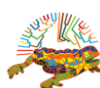
Our research exploits a unique, longitudinal dataset from over 50 years of continuous monitoring of the European badger (*Meles meles*) population at Woodchester Park, coupled with systematic screening for *Mycobacterium bovis*, to investigate the drivers of bTB epidemiology - the most politically, economically, and socially significant wildlife disease in the UK, in wildlife hosts.

We combine whole-genome re-sequencing of the entire Woodchester Park badger

population across five decades with cutting-edge quantitative genetic approaches to estimate genetic variation in susceptibility, disease progression, and transmission potential. We furthermore explore the roles of inbreeding, gene flow, and evolution, and integrate social network analysis and biomarkers of ageing to assess how behavioural and physiological components of senescence contribute to individual heterogeneity in susceptibility and transmission.

By integrating genomic, behavioural, physiological, ecological and life history data across half a century our project provides a rare window into the evolutionary ecology of infectious disease in wildlife - informing both fundamental evolutionary biology and the practical application of disease control in multi-host systems.

Find us at the conference if you would like to be involved in the project – we have several postdoc positions available and would be excited to support fellowship applications within the framework of this NERC Large Grant (2025-2030).



Inferring genome-wide patterns of ancestral divergence and introgression in genus *Epinephelus*

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POSTER SESSION 1 - P01.353, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Understanding how diversification and speciation occur in the highly connected marine environment, characterised by extensive gene flow through ocean currents and larval dispersal, remains a central question in evolutionary biology. Hybridisation and introgression are recognised as important drivers of speciation, yet their prevalence and role in shaping ancient divergence events in marine taxa remain underexplored. The genus *Epinephelus* of the grouper family (Epinephelidae) presents a compelling system to address these questions due to its species richness, overlapping distributions, unique life history traits such as broadcast spawning and multi-species aggregations, functional hermaphroditism, and documented instances of hybridisation. This study focuses on Clade F and its subclade F3 to investigate patterns of past speciation,

genomic divergence, and potential introgression. Whole-genome resequencing of 14 species revealed discordant, time-calibrated phylogenetic topologies in ancestral lineages during the Miocene and Pliocene, likely reflecting rapid diversification and incomplete lineage sorting. Additionally, we identified widespread genomic signals of introgression in Clade F3, with admixture proportions ranging from 1.67% to 6.78%. The genetic transfer was found to be predominantly directional, moving from deep-water continental shelf lineages to shallow-water coastal species, although the evolutionary implications remain uncertain. This study advances our understanding of marine speciation and positions *Epinephelus* as an example for investigating how hybridisation and introgression influence biodiversity in highly connected oceanic systems.



Inferring the Dry Season Ecology of Malaria Vectors from Allele Frequency Dynamics

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POSTER SESSION 3 - **P03.338**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Malaria vectors, including members of the *Anopheles gambiae* s.l., exhibit strong seasonal fluctuations that their sizes can reach very low in the dry season then rebound rapidly with the first rain, often by multiple orders of magnitude. Aestivation and long-distance migration are the two main hypotheses to account for their persistence, but the sampling of aestivators or immigrants are known to be notoriously difficult, meaning direct evidence is lacking to pinpoint the exact mechanism. Previously we successfully built a mathematical model to estimate the number

of breeders and aestivators from temporal allele frequency dynamics. We extend the idea to propose similar models for long-distance migration as well as a hybrid one containing both processes. These models are fitted to various *An. gambiae* s.l. genomic datasets collected from Africa, and the differences in their preferred persistence mechanisms across species and landscape will be discussed. Understanding the vectors' dry season ecology and population histories is key to the parameterisation of further modelling on biocontrol strategies.



Inner ear asymmetry in birds and mammals: Clues to developmental instability and evolvability

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POSTER SESSION 3 - P03.345, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Mammals may owe part of their evolutionary success to an increased evolvability of their ear. The incorporation of jaw elements into the middle ear has enhanced the genetic, regulatory, and developmental complexity of the mammalian ear, potentially providing greater flexibility for the independent evolution of its functional components. The mammalian ear may thus be more evolvable than the simpler ear structure of other vertebrates, such as birds with one auditory ossicle and a non-coiled cochlea, hence enabling adaptation to diverse ecological niches.

Very little research exists regarding the extent to which properties of the developmental system have contributed to the evolvability of the mammalian ear. The higher genetic and developmental complexity in mammals may increase the risk of developmental instabilities, which can be assessed through the study of bilateral asymmetry. In contrast, the fewer genetic and developmental factors involved in avian ear development may limit its evolutionary potential, while simultaneously reducing the risk of developmental disorders.

This study focuses on the patterns of directional and fluctuating asymmetry in the mammalian and avian inner ear. We test the hypothesis that mammals exhibit greater fluctuating asymmetry in ear shape than

birds, reflecting potential instability related to increased developmental complexity. Using geometric morphometrics, we compare intra-specific patterns of asymmetry across four species: two birds (*Passer domesticus* and *Buteo buteo*) and two mammals (*Homo sapiens* and *Sciurus vulgaris*), each with sample sizes of 20-30 individuals. Our results indicate that the squirrel sample exhibits the highest magnitude of fluctuating asymmetry, while the other three samples show similar, lower levels. Interestingly, directional asymmetry contributed more substantially to the overall asymmetry pattern in the squirrel sample, and, although to a lesser extent, in the human sample. In both mammals, the asymmetry patterns appear to be primarily driven by shape differences in the cochlea between the left and right inner ear. This may suggest that the shape of the semicircular canals, responsible for balance and posture, needs to be more constrained, especially in the squirrel, an agile species that relies on precise and rapid movements. In contrast, directional asymmetry was minimal in both bird samples. The asymmetric patterns observed in the mammalian cochlea are likely due to its complex, coiled morphology, a feature absent in birds, whose simpler inner ear structure may account for the lack of a comparable asymmetry signal.



Insights into the genome of dromedary camels: signatures of positive selection and copy number variations

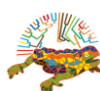
Bahbahani H¹

¹Kuwait University

POSTER SESSION 1 - P01.346, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The genome of dromedary camels has been targeted by different evolutionary forces that shaped its diversity pattern. These forces are mainly attributed to the historical utilization of dromedaries in transportation and trading, as well as their adaptation to the harsh environmental conditions they experience. In this research project, we have investigated the genome of dromedary camels for signatures of positive selection, and built the first dromedary copy number variation map using whole genome sequence data. Using decorrelated composite of multiple signals (DCMS) approach, a total of 36 candidate regions were identified to be under positive selection. These regions overlapped with 185 haplotype blocks encompassing 1,340

haplotypes, of which 30 (~2%) were found to be approaching fixation. Utilizing four CNV callers employing read-depth, split-read and paired-end mapping approaches, 2,557 regions, categorized as 1,322 losses, 122 gains, and 1,113 “mixed regions” comprising both types were called covering 1.16% of the dromedary autosomes. The defined candidate selection and CNV regions harbor genes associated with different biological processes related to the dromedaries’ adaptive physiologies, including neurological pathways, musculoskeletal development, fertility, fat distribution, immunity, visual development, and kidney physiology.



Integrating gene expression and biochemical approaches for understanding the interactions between the antibiotic enrofloxacin and environmental stressors in the Mediterranean mussel (*Mytilus galloprovincialis*)

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POSTER SESSION 3 - P03.308, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

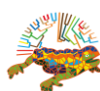
Background: Anthropogenic activities like pharmaceutical pollution increasingly drive alterations in marine coastal ecosystems. The widespread use of fluoroquinolone antibiotics, such as enrofloxacin (ENR), raises concerns due to their accumulation in aquatic environments and potential adverse effects on non-target organisms. However, there is a notable lack of comprehensive information regarding the impacts of antibiotic contamination under a climate change scenario on marine organisms, such as bivalves. Furthermore, there is limited understanding of the ability of bivalves to restore their cellular and biochemical homeostasis following exposure to these stressors. This study aimed to assess cellular and transcriptional responses in the mussel *Mytilus galloprovincialis* exposed to environmentally relevant concentrations of ENR under ambient (20°C) and predicted global-warming temperatures (25°C). Responses were evaluated in the mussels' gills after 7 (EXP7) and 14-day (EXP14) exposure periods, followed by a 14-day (REC) recovery period.

Materials and methods: Mussels (50-60 mm shell length) were previously acclimated in filtered natural seawater (FNSW) at 20 ± 1 °C and salinity 30 ± 1 for 14 days. After the acclimation period, mussels were exposed to 5 and 500 ng/L ENR under ambient and increased temperature, and tissues (gills) were collected after EXP7, EXP14, and at the end of the recovery period (REC). A total of 15 mussels per treatment were randomly selected among the aquaria and divided as follows: 5 whole mussels were used for chemical quantification. Gills from 5 mussels were used for gene expression analysis, and other 5 gills were used for biochemical analyses. Biochemical analyses were as follows: electron transport system activity, ETS; carbohydrates, CARB; proteins, PROT; lipids, LIP; acetylcholinesterase, AChE; DNA single-strand breaks, DNAssB; superoxide dismutase, SOD; glutathione-S-transferase, GST. , Gene expression patterns were analyzed in nine genes: ATP-binding

cassette B, abcb; CYP4Y1, cyp4y1; Caspase, casp8; Tumor Protein p53, tp53; Piruvate Kinase, pkpyr; Isocitrate Dehydrogenase, icdh; Catalase, cat; Superoxide Dismutase, sod; Glutathione S-transferase, gst).

Results: The mussels exposed to ENR showed overexpression of (sod), Catalase (cat), and Caspase genes (casp8) for all temperatures and ENR concentrations. ENR concentration significantly affected the expression of abcb, cyp4y1 and gst. pkpyr and tp53 showed a gene expression regulated by exposure time, with a downregulation after the first seven days and an upregulation after 14 days of exposure. An underexpression of almost all genes characterized the recovery period. These results suggest increased oxidative stress and detoxification responses and a potential compromise of energy metabolism due to ENR exposure over time. Biochemical analyses showed a depletion of energy reserves (mainly lipids and proteins). GST activity increased after EXP14, and a dysregulation of GST activity persisted after the recovery period. The DNA integrity was affected by both ENR concentrations.

Conclusions: The observed gene expression responses indicate that mussels initially activate defense mechanisms, while prolonged ENR exposure progressively weakens their detoxification capacity. The reduced expression of key detoxification genes during the recovery phase suggests that the antibiotic's effects may persist over time. The observed depletion of energy reserves could exacerbate this trend. The detection of potential DNA damage and the dysregulation of the transcription factor tp53 need to be deeply investigated. Combining expression patterns with physiological responses may provide valuable insights into the interactions between emerging contaminants and environmental stressors, highlighting the long-term ecological impacts of antibiotic pollution in habitat-forming species like mussels.



Integrating thermal physiology and evolutionary ecology to understand biodiversity responses to climate change in Brazilian ecosystems

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POSTER SESSION 3 – P03.215, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background. Rapid environmental changes, including climate and land use change, profoundly impact biodiversity in tropical ecosystems. At the Laboratory of (Bio)Diversity in the Anthropocene (BioDivA), we integrate thermal physiology, evolutionary ecology, and landscape ecology to investigate the effects of these changes on species' physiology, ecological interactions, and evolutionary trajectories across diverse Brazilian habitats. Materials and Methods. We examine thermal responses and climate-induced stress in amphibians, reptiles, and birds, analyze the consequences of urbanization and habitat modification on community structure and species interactions, particularly in pollinator assemblages, and assess the impacts of infrastructure development on local biodiversity. Additionally, we incorporate population-level analyses integrating past, present, and projected future data to

understand demographic and evolutionary responses to climate change. Behavioral plasticity and adaptive responses to environmental stressors are also addressed, alongside the effects of fire and landscape disturbance on endemic and threatened species. Results and Conclusions. Employing phylogenetic frameworks, experimental approaches, and extensive fieldwork, our multidisciplinary research aims to elucidate the mechanisms underlying species resilience or vulnerability to environmental change. These findings are essential for informing conservation strategies and adaptive management in biodiversity hotspots subject to rapid anthropogenic pressures, contributing to a broader understanding of evolutionary responses to global change and highlighting the importance of integrative approaches in addressing conservation challenges.



Interplay between sex determination and sex chromosome turnover in Sepsid flies

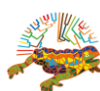
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¹Institute Of Science And Technology Austria

POSTER SESSION 3 - P03.343, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Sex chromosomes, such as X and Y pairs, arise from a pair of regular autosomes, but over time can become highly specialized fully differentiated sex chromosomes. Such fully differentiated sex chromosomes are typically very stable, as their reversal to autosomes would be deleterious. The insect order Diptera (flies and mosquitoes) represents a striking exception, as it has acquired many novel sex chromosomes, with the ancestral X reverting to an autosome. How turnover occurs at the molecular level is poorly understood, as sex determination mechanisms have only been elucidated in a handful of dipteran species, such as the model organism *Drosophila melanogaster*, the disease-carrying mosquitoes of *Aedes* and *Anopheles* genus and the agricultural pest *Ceratitis capitata*.

To bridge this gap, we investigated sex determination in a Sepsid fly, whose X chromosome is the result of a turnover event, but whose sex determination mechanism is completely unknown. We first obtained genomic sequences from male and females, allowing us to characterize X- and Y-linked sequences. We then combined this genomic analysis with an extensive gene expression dataset of early embryogenesis, the stage during which sex determination is set up, to obtain a set of high quality candidate genes for sex determination. These findings shed new light on how sex determination causes and in turn is shaped by sex chromosome turnover, and provide a deeper understanding of reproductive biology in a highly diverse clade such as Diptera.



Intra-specific variation in aging and its life history implications in African annual *Nothobranchius* killifish: an experimental study

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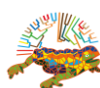
POSTER SESSION 1 - P01.206, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Harsh environments lead to unique adaptations, as seen in African annual *Nothobranchius* fish that live in temporary pools in the East African savannah. The unpredictable conditions in their habitats are directly linked to their adaptations and life history trade-offs. Hence, certain phenotypic traits in different populations may be influenced by the specific lifespan expectancy of each group based on the duration of their temporal habitats. Populations in drier regions are expected to have a faster life strategy and age more quickly compared to those in wetter regions. To investigate if the lifespan of different populations predicts rates of demographic, functional, and reproductive aging, we conducted a common garden experiment using F1 generation fish from two

populations of four species, each representing dry and wet regions.

Our analysis confirmed that fish from dry regions have a shorter lifespan and experience faster aging, including a more rapid increase in mortality rate, a quicker decline in fertility, and greater cellular and physiological deterioration. Despite being larger at birth and remaining larger until sexual maturity, short-lived fish did not exhibit the pace-of-life syndrome or increased investment in reproduction.

Our findings suggest that differences in lifespan among natural populations are genetically determined and linked to functional and reproductive declines. This challenges the traditional evolutionary theory of aging, which proposes that increased extrinsic mortality leads to shorter lifespans and faster aging.



Intraspecific variation in mate attraction shapes hybridization in a young species group

Spirina M¹, Beele L¹, Van Reempts E¹, Mortier F^{1,2}, Auger P³, Wybouw N¹

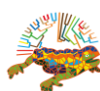
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POSTER SESSION 3 - P03.258, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Speciation is often driven by pre-mating isolation barriers that prevent interspecific mating. Although these isolation barriers can evolve rapidly in natural populations, these barriers are often incomplete, leading to interspecific hybrids. Despite the importance for our fundamental understanding of speciation, the specific mechanisms that regulate the formation of interspecific hybrids remain poorly understood in many systems, especially in speciose mite taxa.

In our lab, we study several features of the mating behaviour and hybridization patterns of three Tetranychus spider mite species. No-choice genetic crosses using a wide panel of near-isogenic lines revealed varying and asymmetrical degrees of hybridization. Using the same mite lines, behavioural assays uncovered strong intraspecific

variation in mate attraction in one of the focal Tetranychus species. By long-term acclimation to different host plants, we tested whether this attraction covaries with fitness. Quantifying the strength and direction of pre-mating isolation among our three hybridizing species uncovered complex patterns of non-assortative, assortative, and disassortative mating behaviour. We further observed that intraspecific variation in mate attraction is a major genetic determinant regulating interspecific mating. To test whether disassortative mating evolved by reproductive interference, we included an allopatric Tetranychus species in our behavioural assays. Together, our findings contribute to a broader understanding of how behavioral mechanisms can shape hybridization.



Introgression and divergence in a young species group

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POSTER SESSION 1 - P01.013, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: The process of speciation concerns often not only pairs of species but rather groups of diverging and interacting taxa. Hence, to understand the evolution of species' diversity and their persistence, it is crucial to understand how gene flow and evolution of reproductive isolation shape whole species complexes. Hybridization and repeated backcrossing eventually lead to introgression, whereby genetic material is transferred into the gene pool of another species. An understanding of the genomic signatures left by beneficial and deleterious introgression, and the barriers that impede it, can help identify the genomic regions important for adaptation and speciation. Unanswered questions remain about how introgression influences speciation, especially in groups of young taxa.

Materials and Methods: Using a comparative population genomic framework, we utilise whole-genome resequencing data from five closely related *Formica rufa* group wood ant species that live in sympatry (altogether 93 individuals). We construct a species tree, analyse population structure, estimate nucleotide diversity and genetic differentiation and divergence, and infer introgression, aiming to disentangle genomic patterns of divergence and introgression – how different processes have contributed to *Formica* speciation during their recent divergence history.

Results: We revise earlier mitochondrial phylogenies with a nuclear genomic tree, and demonstrate that introgression occurs in line with observed natural hybridisation, both between sister and non-sister species. We find genome-wide correlations between divergence, differentiation, and diversity that are in line with theoretical expectations for young species. Despite earlier evidence for

polygenic species barriers in *Formica*, we find no genome-wide correlation between differentiation and divergence that would be expected under a model of polygenic barriers – likely explained by a dominating effect of ancestral diversity in young species. Likewise, although hybridisation has been shown to have deleterious consequences for *Formica*, we find no strong positive correlation between introgression and recombination, suggesting introgression doesn't have a predominantly deleterious effect. Instead, regions with high introgression show low diversity, suggesting selection has locally favoured introgression. This could be due to sharing of adaptive alleles or reduction of genetic load. Interestingly, we show that gene flow in *Formica* could potentially cross multiple species boundaries even in the absence of direct interbreeding between each of the species.

Conclusions: When hybridization and introgression have both benefits and costs, their long-term impacts are challenging to predict. Our findings shed light on their role in a species complex with previous evidence for both polygenic gene flow barriers and deleterious fitness consequences of hybridisation but also potential adaptive benefits of introgression: previous research suggests that introgression could help these species cope with genetic load or facilitate their survival in a changing climate. Further investigation on the signatures of adaptive introgression will shed light on its evolutionary impacts. This work underscores that divergent taxa may gain by not completing speciation, although the long-term impacts of introgression are complex to predict.



InUrFaCE - Initiative of Urban Facades Creature Exposition

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POSTER SESSION 1 - P01.132, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Biological diversity encompasses the diversity of all animal and plant species, the diversity of all ecosystems and the genetic diversity within species. It is the basis of human existence, providing food, health and well-being. In recent decades, the rate of biodiversity loss has accelerated dramatically as a result of human activities. Unfortunately, despite the escalating situation, public awareness of the ongoing biodiversity crisis is minimal. The registered association InUrFaCE therefore tries to address this future-relevant topic artistically and to raise people's awareness on an emotional level in order to increase the appreciation of biological diversity in society and the willingness to protect it. As positive emotions are more likely to motivate action, these are to be evoked through beauty and wonder.

Materials and Methods: An interdisciplinary collaboration between established street artists and scientists in the field of biodiversity research resulted in painted facades that 'jump in the face' of the public to raise awareness of the problem of biodiversity loss. An 'artists/scientists' workshop organised by InUrFaCE took place at the Leibniz Institute for the Analysis of Biodiversity Change (LIB) in Bonn, Germany. During a guided tour behind the scenes, the artists were given an insight into the work of the scientists and an overview of the diversity of organisms. The artists and scientists worked together on ideas as to how the project can find its place in the public in the form of so-called murals. This workshop was

medially documented (film and photos) and the artists and scientists were interviewed during the workshop. During the painting of the mural, a small information stand accompanied the project to inform passers-by/visitors about the project and to arouse their curiosity. In addition, an information sign with a QR code was installed on the mural, allowing any visitor to directly access information about the project and the organisations involved.

Result: Last year, two successful projects were carried out in Bonn in collaboration with the LIB, with the support of the Dr. Hans Riegel-Foundation (pilot project 1 Bonn's old town) and with the support of Deutsche Bahn and go.Rheinland (project 2 at the UN Campus train station). The two projects were realised by the artists Roman de Laporte (Jack Lack) and Kai Niederhausen (Semor). These two projects can be viewed on the following website: <https://art-meets-biodiversity.com/>. For the pilot project we also made a short documentary about the workshop and the work on the wall: <https://www.youtube.com/watch?v=avol4ZGO82o&t=80s>

Conclusion:

This collaboration and form of presentation reached an unprecedented audience. Our institutes/museums generally reach a very small audience with the topic of biodiversity, which rarely goes beyond the circle of experts/informed people. The artists have a huge reach through their presentation in public spaces and through their platforms on the internet (artist websites, Instagram, etc.).



Investigating mitonuclear interactions in hybridizing newt species *Triturus ivanbureschi* and *T. macedonicus*

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POSTER SESSION 3 - P03.309, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Mitonuclear coadaptation involves coevolution between species-specific sets of nuclear genes and the mitochondrial genome. Interspecific hybridization may disrupt co-adapted gene sets, potentially leading to mitonuclear incompatibility in hybrids, causing lower fitness and possibly contributing to reproductive isolation. Natural hybrid zones provide an opportunity to explore the dynamics of coadapted gene complexes in the face of interspecific gene flow. The newt species *Triturus ivanbureschi* and *T. macedonicus* hybridize in the Balkan Peninsula with large-scale introgression of *T. ivanbureschi* mtDNA into *T. macedonicus*, allowing for an assessment of nuclear gene flow that could mitigate the effects of mitonuclear disruption.

Materials and methods: We assessed nucleotide divergence from mitogenomic and transcriptomic data available for *T. ivanbureschi* and *T. macedonicus* in mtDNA-encoded genes (12 genes), nuclear genes interacting with mtDNA (Nmt, 138 genes) and nuclear genes non-interacting with mtDNA but expressed in mitochondria (N, control, 239 genes). Then, we genotyped a subset of species-specific nonsynonymous SNPs in the nuclear genes using molecular Inversion Probes (MIP). A total of 166 samples of *T. ivanbureschi* and *T. macedonicus* which covered hybrid zone populations and genetically pure populations as a reference, and together with mtDNA type, were used for genomic cline analysis.

Results: Analysis of nucleotide divergence showed a significantly higher value for mtDNA-encoded genes (mean dxy = 0.074, p = 0.024, pairwise Wilcoxon), while for Nmt (mean dxy = 0.007) and N genes (mean dxy = 0.008) it was lower by an order of magnitude. Despite a lack of significant differences between groups, we

observed a similar tendency for rate of nonsynonymous substitutions, with the highest value for mtDNA-encoded genes (mean dN = 0.013) and much lower results for Nmt (mean dN = 0.003) and control genes (mean dN = 0.004). After strict quality control and filtering, a total of 46 genes were used for cline analysis (10 Nmt and 36 N control genes). Introgression of two genes: coenzyme Q3, a critical component of the electron transport pathways, and 12S mitochondrial rRNA chaperone 1, required for proper assembly of the 28S small mitochondrial ribosomal subunit, departed from introgression of other genes and followed the pattern of *T. ivanbureschi* mtDNA introgression. A total of 7 out of 10 Nmt genes showed restricted gene flow between species (mitochondrial ribosomal proteins, complex I) and for 1 gene (MTERF4) we found evidence of introgression of the *T. macedonicus* allele into the *T. ivanbureschi* genomic background.

Conclusions: We found high divergence in mtDNA between *T. macedonicus* and *T. ivanbureschi* compared to Nmt and control N genes. While no difference between Nmt and N genes in divergence and nonsynonymous substitution rate were found between the species, genomic cline analysis of a subset of genes in natural populations offered direction for further investigation of mitonuclear coadaptation in newts. We identified genes that possibly co-introgress with mtDNA to potentially compensate for the negative influence of mitonuclear mismatch. These genes provide a valuable foundation for future functional and expression studies that could directly test the role of mitonuclear interactions in shaping reproductive isolation.



Iodine deficiency-induced goiter constrains inland colonization of fish

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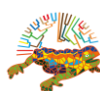
POSTER SESSION 1 - **P01.366**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Iodine is an essential trace element for vertebrates. Iodine is a component of thyroid hormones, which regulate diverse physiological functions. However, iodine is not evenly distributed throughout the earth. The ocean is rich in iodine, whereas iodine is scarce inland. How does spatial variation in iodine availability influence the ecology and evolution of natural animal populations? To address this question, we used two stickleback species: the three-spined stickleback (*Gasterosteus aculeatus*), which has colonized and adapted to diverse freshwater environments, and the Japan Sea stickleback (*G. nipponicus*), which is exclusively marine.

Our common garden experiments revealed that *G. nipponicus* showed thyroid goiter in freshwater mesocosm lacking iodine. Due to iodine deficiency, these fish had low plasma thyroid hormone levels and high expression levels of a thyroid-stimulating hormone (TSH)

gene in the pituitary gland, which likely stimulates thyroid proliferation constitutively. Goiter reduced the foraging efficiency. Goiter was rescued by iodine supplementation. By contrast, *G. aculeatus*, whether marine or freshwater ecotypes, showed no goiter in freshwater mesocosm. Surprisingly, *G. aculeatus* maintained higher plasma thyroid hormone levels and had lower pituitary TSH expression levels than *G. nipponicus*. Our whole body thyroid hormone measurements indicated that *G. aculeatus* can retain iodine and/or thyroid hormones more efficiently than *G. nipponicus* under iodine-deficient conditions.

Thus, cryptic variation in the genetic predisposition to thyroid goiter was revealed in iodine-deficient freshwater environments. This cryptic variation may lead to different abilities of freshwater colonization between closely related species.



Is there a limit to the adaptive potential of chromosomal inversions with regard to global warming?

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POSTER SESSION 1 - P01.356, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Global warming is a fact, and organisms have the following options to avoid extinction: to acclimate (phenotypic plasticity), to adapt through genetic changes or to migrate to more favorable regions. Useful genetic markers to study genetic adaptations are chromosomal inversions. In this context, *Drosophila subobscura* is an excellent model species, because it presents inversions in its whole karyotype. Also, it has been possible to define 'cold', 'warm' and 'non-thermal' adapted inversions, both in the Palearctic and American colonized regions. The aims of this research were to study the differentiation of this polymorphism in two populations from Jastrebac Mt. (Serbia) which differed in habitat conditions (beech and oak forests) sampled in 2023. Also, it was possible to compare changes of this polymorphism over time, comparing both populations with those from 1990, 1993, 1994 and 2023. Furthermore, the CTI (Chromosomal Thermal Index), which measured the thermal adaptation of the whole karyotype was computed and compared among all populations. The possible relation between climatic variables (mean, minimum and maximum temperatures, humidity and

rainfall) and chromosomal inversions was also studied. For the A and O chromosomes, microdifferentiation was observed between beech and oak forests populations sampled in 2023. However, multivariate studies showed that changes over time in chromosomal inversion composition turned out to be larger than those due to habitat conditions. Furthermore, it was possible to observe global warming in Jastrebac Mt., because temperatures increased over time, being this increase even significant for mean and minimum temperatures. Furthermore, using a large set of Serbian populations of *D. subobscura*, relations between temperatures (mainly minimum) and chromosomal inversions were observed through a Multidimensional Scaling analysis. Finally, the CTI index increased over time in Jastrebac Mt. for the period 1990-1994, but a stabilization was observed in 2023, Similar results were obtained in other Serbian populations. One possible hypothesis is that there would be a threshold for the increase in 'warm' adapted inversions, which could not be surpassed, since a loss of fitness would occur.



It all began in the center: genomic phylogeny and ancestral range estimates provide new insights into Sulawesi tarsier diversification

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POSTER SESSION 3 - P03.319, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Sulawesi tarsiers (*Tarsius*) are nocturnal, haplorhine primates native to the Indonesian island Sulawesi and surrounding islets. Originally classified as one species, Sulawesi tarsiers have been delineated into 12 (currently) described species by acoustic, genetic and morphological analyses. Recent phylogenetic findings depict that the mountain or pygmy tarsier (*Tarsius pumilus*) is the sister taxon to all other (lowland) Sulawesi tarsiers, with a split date of about 7 million years before further diversification. Lowland tarsiers in turn are divided into two well-differentiated genetic lineages, both of which are represented by several recognized species. It is assumed that the dozen Sulawesi tarsier species have diversified in situ on the island and in the surrounding area. Rapid diversification including dispersal and vicariance events starting 2.3 mya has produced a complex phylogeographic picture requiring further characterization.

Materials and methods: Using MIG-seq technology, we sequenced about 300 tarsier samples from across mainland Sulawesi at more than 1,500 genetic loci and generated the first genomic-scale phylogeny for the genus. We then time-calibrated our phylogeny to relate paleogeographic information to the timeline of diversification events. We used this information to model ancestral ranges of Sulawesi tarsiers as they relate to the evolving shape of the island from the Miocene through to the present. In addition, we used this genomic data plus data derived from 16 microsatellite loci to assess genetic structure and diversity and to explore demographic scenarios to explain the current structure and distribution of Sulawesi tarsiers.

Results: Our analyses confirmed the two previously described major lineages but also

allowed us to resolve the tips of tree with high confidence. This shed light on previous ambiguity, e.g., the low-supported sister-taxa relation between *T. fuscus* and the far eastern population of *T. dentatus*. Spatial structure analyses further revealed that continuous genetic clines derived from isolation by distance rather than evolution in allopatry seems to explain the genetic uniqueness of this remote *T. dentatus* population. Interestingly, we also found a substantial admixture between *T. spectrumgurskyae* and *T. supriatnai* in a population previously assigned to *T. supriatnai*, illuminating the necessity for further investigation on aspects of secondary contact and hybridization.

Ancestral range estimations suggest that tarsier diversification on Sulawesi started from western Central Sulawesi and not, as assumed previously, from the southeast. This is also in concordance with the presumed expansion of other Sulawesi mammals and supports the notion that area increase led to expansion and diversification from the same region.

Conclusions: We highlight several phylogenetic and biogeographic relationships at the species level that are important for a comprehensive understanding of evolution on Sulawesi and for the conservation of endemic lineages. In addition, our analysis emphasizes the importance of specific geographic areas and historical barriers to tarsier diversification, as well as contemporary contact zones between species. We demonstrate the significance of this study of Sulawesi tarsiers both in understanding and conserving this endemic and unique clade of primates, and in informing a wider understanding of the interspecific and intraspecific genetic variation that follows a burst in diversification.



Latitude Matters: A Global Phylogeographic Perspective on Climate-Driven Demographic Responses in Tarantulas

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POSTER SESSION 3 - **P03.255**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Understanding how climate change shapes biodiversity across the globe is a central challenge in contemporary ecological and evolutionary research. While much research has focused on temperate taxa, empirical studies testing how species from different latitudes have responded to historical climatic shifts at global scales remain scarce. Here, we present the first global phylogeographic analysis of tarantulas (Theraphosidae) that integrates genetic data, species distribution modelling, and bioclimatic reconstructions to investigate how climate change since the Last Glacial Maximum (LGM) has impacted their demographic histories and genetic diversity.

Tarantulas, with low dispersal ability, broad latitudinal distributions, and high endemism, offer a powerful system to test hypotheses on latitude-specific responses to environmental change. Importantly, they are among the most threatened spider groups today, facing intense pressure from habitat loss, climate change, and overexploitation for the pet trade. We compiled mitochondrial DNA data and present-day distribution records for 52 tarantula species spread across different latitudes and continents (Asia, Africa, and the Americas). Demographic responses (Tajima's D and R2) and genetic diversity (Nucleotide diversity) were calculated. Species distribution models were built using present-day bioclimatic variables and projected onto LGM conditions to quantify percent climatic suitability change since the LGM. We tested multiple hypotheses concerning the relationships between latitude, climatic niche dynamics, genetic diversity, and demographic shifts.

Our analyses revealed strikingly latitude-dependent demographic responses. While neither latitude nor climatic suitability change alone explained significant variation in demographic indices, their interaction did. Specifically, at higher latitudes (>32°),

demographic expansion was positively associated with greater climatic suitability gain since the LGM. In contrast, lower-latitude species (<18°) exhibited a weak negative relationship between demographic stability and climatic niche change. Mid-latitude species (18–32°) showed no significant trend. These findings suggest that the demographic benefits of post-glacial warming were predominantly experienced by high-latitude tarantula species, potentially due to glacial retreat and increased habitat availability. Conversely, tropical species may have faced demographic instability as a result of subtle but ecologically disruptive climatic shifts. These results provide rare empirical support for Darwin's and Janzen's contrasting predictions—indicating that both tropical vulnerability and temperate plasticity are evident in ectothermic taxa, depending on latitude.

Surprisingly, we found no significant correlation between genetic diversity and either latitude or climatic suitability change. This lack of pattern suggests that mitochondrial diversity may be decoupled from recent demographic dynamics or that it reflects deeper evolutionary processes beyond the scope of post-LGM climatic shifts.

Together, these results offer the first macroecological evidence for geographically structured demographic responses to late Quaternary climate change in a globally distributed invertebrate group. By demonstrating a significant latitude-dependent interaction between niche change and demographic trajectories—but not genetic diversity—our findings highlight the complexity of climate change effects across spatial scales. As climate change accelerates in the Anthropocene, understanding such heterogeneous responses is critical for forecasting biodiversity loss and informing conservation strategies, especially for ecologically sensitive and understudied taxa like tarantulas.



Leveraging macrogenetics to understand global patterns of intraspecific genetic variation on genes under purifying selection

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POSTER SESSION 3 - **P03.342**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Understanding the processes that govern multispecies intraspecific genetic diversity is lacking - macrogenetics seeks to change that. In its short history, macrogenetics has offered essential insights into the impacts of climatic and anthropogenic changes in multispecies genetic diversity, guiding conservation policy and planning. However, most macrogenetic studies are on neutral or mitochondrial loci and thus, global patterns in functional genetic diversity remain poorly understood. Here we use abundant publicly available sequence data from candidate immune genes, specifically toll-like receptor (TLRs), to explore the most important predictors of functional multispecies genetic diversity across birds and mammals. Unlike commonly recognizable immune genes such as the MHC, which are under balancing or positive selection, TLRs are involved in an organism's innate immune system and are under purifying selection. TLRs are thus a good model for understanding how populations maintain deleterious mutations

and how purifying selection contributed to genetic diversity patterns. The strength of purifying selection is linked to effective population size (N_e). Across a preliminary dataset of passerines (7 species, 600 individuals) we observed a relationship between census size (proxy N_e) and genetic diversity (π) ($r = 0.50$, $p = 0.05$), but the negative association between census size and the ratio of nonsynonymous to synonymous polymorphisms and was not as strong as expected ($r = -0.26$, $p = 0.26$), suggesting that some source of variation remains unexplained. We are now expanding the preliminary dataset and exploring if species' life history traits and geography could contribute to TLR multispecies genetic diversity and the efficacy of purifying selection. By answering these questions, we hope our results will provide new insights on how species traits, selection and drift associate with functional multispecies genetic diversity and mutational load.



Loud environment and noisy neighbors: impact of urbanization and interspecific competition on song characteristics of Blue and Great tits

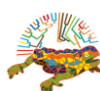
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POSTER SESSION 3 - **P03.350**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Birds use acoustic communication to attract mate and defend their territory, making it very sensitive to selection pressures. Environmental factors, such as ambient noise, can exert strong ecological pressure by masking or altering signals. Anthropogenic noise, characterized by low frequencies, may reduce acoustic niches available for birds. With the advent of urban ecology, it has been showed that several passerine species sing at higher minimum frequencies in response to acoustic pollution in cities. In parallel, interspecific competition may also lead to a decrease in acoustic niches, that might result in character displacement. Blue tits (*Cyanistes caeruleus*) and Great tits (*Parus major*) have overlapping song characteristics in allopatry but this overlap is reduced by the add of a trill in the blue tit song, which reduces territorial response of Great tits (i.e. reduced interspecific interference). In this study we wanted to investigate how these two selection pressures (ie noise and interspecific competition) affect song characteristics when applied together. We recorded Blue and Great tits (218 and 240 respectively) in Dijon and Besancon (eastern

France), as well as in two nearby forests, allowing us to have gradients of acoustic pollution and interspecific competition, from 2022 to 2024. We showed that both acoustic pollution and interspecific competition affect song frequency but only in Great tits, that sing higher in noisy environments and lower in high interspecific competition context (i.e. high bleu tit density). Even if Blue tits are considered the dominated species, they do not seem to change the spectral or temporal characteristics of their song. To investigate whether these changes on Great tits song affect territorial response in Blue tits we conducted two playback experiments in spring 2025 in urban and forest habitats , on the same studied sites described above. We used songs of Great tits emitted i) in noisy and quiet environments and ii) in high versus low density of Blue tits. We expected Blue tits to react more aggressively to Great tit songs from places with i) high acoustic pollution and ii) low blue tit density, because of their higher frequency . Our results highlight the importance of considering how different ecological forces shape communication signals and how it impacts territorial behaviours.



Low Genetic Diversity of Key Immune Genes in the Critically Endangered Burrunan Dolphin (*Tursiops australis*)

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POSTER SESSION 3 - **P03.274**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Disease in cetaceans is an important factor contributing to population decline. Burrunan dolphins (*Tursiops australis*), are a newly recognised species with two resident populations in Victoria, Australia: Gippsland Lakes (GL) and Port Phillip Bay (PPB), with small genetic effective population sizes (PPB: 81.5, GL: 65.5). The GL population of Burrunan dolphins experienced two mass mortality events due to Fresh Water Skin Disease (FWSD) in 2006 and 2020. In the recent mortality event, 12 dolphins were confirmed deceased and 50% of the population is presumed deceased. It is critical to investigate the species' immune gene repertoire as a factor influencing disease outcomes. A key region of the genome that is targeted in conservation genetics is the Major Histocompatibility Complex (MHC), a family of adaptive immune genes. MHC genes code for receptors that are responsible for detecting pathogens, thus diversity within these genes is directly related to immune fitness. MHC is highly polymorphic since it undergoes birth-death evolution, giving rise to duplication and loss of loci within the genome. The aim of this research is to investigate the genetic diversity of MHC across taxa in the *Tursiops* genus, as well as within Burrunan dolphins' populations to determine their immune fitness.

MHC class I exons two and three from 93 dolphins, including Burrunan dolphins (n = 77, GL n = 33, PPB n = 37, other locations n =

7), common bottlenose dolphins (*T. truncatus*) (n = 11) and Indo-Pacific bottlenose dolphins (*T. aduncus*) (n = 5) were sequenced using the Illumina MiSeq Platform. The absolute number of putative alleles was measured across species and populations. The individual-level diversity (indicative of levels of homo- and heterozygosity) was measured according to number of alleles per individual and compared across species and populations. Using a combination of short and long amplicons we also determine the genomic structure of MHC within the Burrunan dolphin genome, and the possible presence of copy number variation within the species. This analysis revealed that Burrunan dolphins have significantly reduced diversity than the other species, with fewer absolute alleles and fewer alleles per individual. There was also a significant difference when comparing Burrunan dolphin populations, wherein GL had lower diversity than PPB, and indicators of high levels of homozygosity, a trait of inbred populations. The reduced genetic diversity may be contributing to poor health outcomes for Burrunan dolphins, particularly those in GL. This research has generated critical information contributing to the heightened need for conservation and management of a highly threatened species. We also provide important information for the immunological evolution of species within the *Tursiops* genus.



Methylome and transcriptome responses to hydrocarbon pollution: acute and priming effects

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POSTER SESSION 1 - P01.253, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Environmental stress either from natural or anthropogenic sources are key features organisms must navigate to survive and reproduce. Environmental stressors therefore can elicit strong plastic physiological and behavioural responses in organisms. Chemical stressors, especially anthropogenic ones such as hydrocarbons, are some of the most potent, possessing genotoxic, mutagenic, carcinogenic and cytogenic effects. By quantifying and understanding how organisms plastically respond to intense chemical stressors we gain a better understanding of phenotypic plasticity and will be able to plan more effective recovery and conservation efforts. Exposure to a stressful event can lead to an acute response, buffering or mitigating the effects of stressors, underpinned by changes in gene expression and regulation which are quite widely studied. Additionally, an initial stressful event may change how an organism responds to subsequent stressors ("memory" or "priming" effects) which could provide vital protection for organisms, but priming effects are less well understood. Here we explored the acute and primed effects of exposure to diesel (a hydrocarbon)

in clones of the beadlet anemone *Actinia equina*, assessing changes in DNA methylation and gene expression across the whole genome to understand the epigenetic and phenotypic changes that underpin anemones' responses to pollution. Acute exposure resulted in a wide and novel expression response which was characterised by the enrichment of oxidative stress, xenobiotic detoxification, metabolic shifts, and other stress-related pathways. This was accompanied by an epigenetic shift which somewhat correlates to the expression changes. Primed responses to diesel exposure revealed a subtle but unique response, showing enrichment for pathways associated with metabolism and protein quality control. We therefore demonstrate how *Actinia equina* mounts a multi-faceted response to hydrocarbon pollution, suggest DNA methylation is not key for altering expression changes, and shows how primed responses are subtly different to acute responses. We highlight key pathways and genes for future work to investigate further.



Microsatellite identification of ramet genotypes in a clonal plant *Iris pumila*

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POSTER SESSION 3 - **P03.297**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Iris pumila L. is a perennial species that reproduces sexually and asexually by underground rhizomes (clonal growth). Clonal plants form spatial networks of ramets that may include: a) groupings of individuals of different genetics that have arisen from seeds, i.e. by sexual reproduction; b) groupings of individuals that originate from the clonal growth of a single genet; or c) groupings of individuals that have arisen from both sexual reproduction and clonal growth. For this reason, when sampling individuals in the field, it is difficult to determine with certainty whether the sampled plants belong to the same or different genotypes. *I. pumila* also exhibits significant genetic polymorphism in flower colour, which could be used to identify genetically distinct clones in the wild.

Since plant mating occurs more frequently among neighboring individuals, the question of whether clonality affects mating should depend on how clonal growth affects the spatial relationships between ramets of different genotypes. The plants of *I. pumila* follow the "phalanx strategy" of clonality and form a compact structure of closely spaced ramets. The close grouping of ramets around the parental shoot could potentially limit the intermixing of ramets from different clones. These restrictions on the mixing of clones could have important implications for mating and fertility.

Currently, several techniques using molecular markers allow the unambiguous definition of individual genes and have been used with great success for clonal plants. Highly polymorphic genetic markers offer excellent opportunity to gain new insights into the functional interactions between sexual and clonal reproduction in plants.

The original premise of experimental research was that it is possible to distinguish plant clones by analysing microsatellite loci, i.e. by detecting

the so-called "multilocus allele phenotypes". If a difference between individuals is detected at any locus of a microsatellite marker, we can conclude that the individuals are genetically different and do not represent the same clone. We have assumed that groups of ramets that are very close to each other mostly represent parts of the same clone (genetically identical ramets), while more distant ramets could be parts of different genotypes, i.e. clones.

As no information, to the best of our knowledge, on the use of molecular microsatellite markers in the study of *I. pumila* could be found in the literature, data on the expression of microsatellite markers (EST-SSR) in transcriptomes of other *Iris* species were used. The selection of EST-SSR was based on whether the markers were expressed in closely related species and whether they showed polymorphism. The final analysis was performed with 5 microsatellite loci. In the overall analysis, more than 40 alleles were detected, of which only one is monomorphic and non-informative. The polymorphism levels of all markers generally proved to be high and sufficient to distinguish the individuals, with the exception of a single marker whose amplification revealed only four different alleles.

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Minimal effects of starvation on pre- and post-copulatory male sexual traits in *Drosophila melanogaster*

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POSTER SESSION 3 - **P03.332**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

In polyandrous mating systems, sexual selection continues post-copulation, with sperm from different males competing in the female reproductive tract to determine paternity.

Ejaculates, which consist of sperm as well as seminal fluid, have significant costs associated with their production. Theory and empirical studies show that males must strategically produce and allocate ejaculates based on their socio-sexual environment. However, a caveat of most existing empirical studies is that they have been conducted in benign environments where individuals experience no additional stressors. Stressors are likely to amplify the costs associated with the production of ejaculates, potentially leading to trade-offs in relative investment between pre-copulatory traits, post-copulatory traits, and survival.

To address this problem, we initially studied the effects of starvation on pre-copulatory traits. We found significant negative effects of male starvation on probability and latency of mating, when males were paired with previously mated females. However, unmated females showed no preference for male quality. Additionally, in pairings with previously mated females, we found significant interactive effects of male quality and mating substrate on mating dynamics, with previously mated females preferentially

mating with unstarved males in the presence of food supplemented with live yeast.

Next, we studied the interactive effects of starvation and social competition on post-copulatory traits. Results from these experiments show that starvation has no effect on several post-copulatory traits, including (i) mating duration, (ii) 24hr offspring count, and (iii) remating latency. This is surprising as both sperm and seminal fluid have significant costs associated with their production in *Drosophila melanogaster*. One possible explanation is that while starvation likely has significant negative effects on ejaculate production, theory predicts that terminally stressed males should invest maximally in immediate reproductive opportunities, potentially leading to males strategically transferring higher amounts of available ejaculates. In future experiments, we would like to explicitly test: (i) if starved males are strategically transferring higher amounts of available ejaculates, and (ii) if starved males are preferentially investing in reproduction, and whether this investment comes at the cost of survival.

Overall, our results add to the growing body of evidence showing the complex effects of stressors on reproductive traits, further highlighting the necessity of testing sexual selection in complex environments.



Mitochondrial Haplotype Distribution and Association with Reproductive Mode in Wild New Zealand Mud Snail

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POSTER SESSION 1 - **P01.135**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

P. antipodarum, the New Zealand mud snail, is a model system used to study the “queen of problems” in evolutionary biology: the maintenance of sexual reproduction. This species exhibits two distinct reproductive strategies: diploid individuals reproduce sexually, while triploids reproduce asexually via apomixis. This makes them a powerful model to explore fundamental evolutionary questions about the prevalence of sexual reproduction.

A previous study in 2013 reported a mitonuclear discordance between *P. antipodarum* from different lakes in New Zealand. Authors (Paczynski et al 2013) found a particular mitochondrial haplotype, 1A, to be strongly associated with asexual, triploid snails across different lakes. This was a first discovery of this haplotype and the study was not designed to examine the pattern in detail. Nevertheless, the prevalence of one mitochondrial haplotype is of particular interest, given that each lake typically exhibits its own distinct population genetic structure. Yet, the 1A haplotype in mitochondria seems to be widespread across New Zealand. One hypothesis to explain this would be that 1A is a meiosis distorter, but in order for this to be true, it should be found in low frequency also in the

sexual population. We have set out to collect a large sample of wild snails, focusing particularly on populations at the South Island of New Zealand, with the goal of extending the previous dataset and especially to have a larger sample size of sexual populations. We collected snails from 50 lakes across South Island (N > 10 000). This sample is the largest and geographically most representative to date for this species and, combined with advanced sequencing technology targeting almost the entire mitochondrial genome, allows for in-depth analysis and can potentially answer the following questions: i) What is the geographical distribution of mitochondrial lineages, and how does it relate to nuclear genetic structure? ii) Is the previously observed association between haplotype 1A and asexuality supported at this broader scale? iii) Are there additional mitochondrial markers strongly associated with reproductive mode?

By integrating large-scale field sampling with advanced genomic tools, we aim at shedding light on the potential genetic and ecological factors contributing to the emergence and spread of the asexual lineages of *P. antipodarum*.



Modeling the effect of risk tolerance on biopesticide resistance evolution & crop damage

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POSTER SESSION 3 - P03.267, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Insect pests are a serious threat to agriculture and can rapidly develop resistance to applied pesticides. Biopesticides are gaining popularity as an alternative to chemical pesticides because of their lower environmental impact and ability to exert more complex selection pressures on pests. These complex selection pressures can aid in long-term sustainable pest management strategies by constraining resistance evolution by exploiting genetic trade-offs. We have previously demonstrated that negative trade-offs between polygenic resistance traits combined with increasing the number of unique biopesticides in the landscape can dramatically suppress resistance evolution on long time scales. Relaxing the intensity of pesticide application can further slow the evolution of resistance, but how much crop damage is economically tolerable? While several models explore the effect of economics on chemical pesticide resistance evolution, few model specifically biopesticide resistance evolution—and its underlying complex polygenic traits—under economic constraints. Furthermore, many models use fixed pesticide application strategies; in reality, growers sometimes do

not adhere to a strict schedule and instead react as needed to high pest densities. More risk-tolerant growers endure higher pest densities before applying pesticides. I investigated the effects of different risk tolerance levels on pesticide resistance evolution using the individual-based model *resevol*. In *resevol*, individual pests have their own virtual genomes, allowing for trade-offs between resistance traits. To simulate risk tolerance, individual farms apply pesticide only upon pest populations surpassing a certain density, with some simulations having a higher threshold than others. I show that increased risk tolerance leads to fewer biopesticide applications and results in overall less resistance evolution. However, more crop damage is expected with more relaxed applications. Increasing the number of unique biopesticides in the system lessens both crop damage and resistance evolution. There is a trade-off between labor costs and resistance evolution, and further metrics are needed to complete a cost-benefit analysis. These results are useful for devising economically and ecologically sustainable pest management strategies and policies.



Molecular mechanisms of colouration using salamanders as a model

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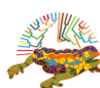
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POSTER SESSION 3 - With Aperitif, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Animal colouration has fascinated evolutionary biologists since the days of Charles Darwin. Despite the wealth of knowledge about the genetic basis of colouration in many species, lots of phenotypic variation remains unexplained. Recent approaches have focused on the role of methylation in colour variation, showing a positive impact of DNA methylation's on colouration. However, the role of RNA methylation is much less understood. In this study, we investigated RNA methylation in the European fire salamander (*Salamandra atra*), known for its diverse colour patterns. In this group, there are typical striped colourations and also morphs lacking stripes. We examined genetic changes on methylation patterns potentially associated with colouration using long-read direct RNA sequencing on three colour morphs—yellow, striped black and yellow, and brown. We created a de-novo transcriptome for alignment (containing ~22,000 genes, ~70% BUSCO) and analysed

gene expression and methylation differences between different colourations. Our findings revealed a small number of differentially expressed and differentially methylated genes, including many associated with pigmentation processes. Notably, genes in the melanin biosynthetic pathway, such as *MLANA*, *PMEL*, *TYR*, and *TYRP1*, were downregulated in yellow skin, whilst *TYRP1* and *PMEL* are also hypomethylated in yellow skin. Furthermore, the genes *TYR* and *TYRP1* are both hypomethylated in brown skin. Additionally, *DGAT2*, a gene related to carotenoid metabolism, was upregulated in yellow and brown skin compared to black. Clearly, fire salamander colouration, and likely amphibian colouration in general, has a variety of molecular mechanisms contributing to its manifestation. Our results provide crucial evidence that RNA methylation is likely involved in the diversity of colouration we see throughout the animal world.



Monogamy may remove constraints on reproductive tissue investment imposed by intense sexual selection

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POSTER SESSION 3 - **P01.105**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

A key factor influencing an individual's reproductive success and fitness is the allocation of resources to reproductive tissue. Previous research has documented positive influences of sexual selection on the evolution of reproductive traits such as testis size. However, whether sexual selection shapes overall reproductive tissue investment patterns is unclear. Moreover, most studies on the relationship between sexual selection and reproductive allocation have focused on males, and little is known about female reproductive tissue allocation in response to sexual selection. In addition, whether the ecological/demographic context modulates these effects is unknown. We used experimental evolution on an insect model system to assess the independent and interactive effects of sexual selection intensity (intense [polygamy] vs. relaxed [monogamy]) and metapopulation structure (absent vs. present) on reproductive tissue investment in both males and females. Counterintuitively, we found that individuals of both sexes evolving under monogamy had higher relative reproductive tissue investment than individuals evolving under polygamy. We found no independent effect of metapopulation structure or any interactive effect with sexual selection on reproductive tissue investment of either sex.

Our results suggest that trade-offs between pre- and post-copulatory sexual selection may constrain reproductive tissue investment under intense sexual selection, and that selection under monogamy removes this constraint. In our selection experiment, not only postcopulatory but also precopulatory sexual selection was removed in monogamous populations. That is, in monogamous populations, female choice and the need for males to compete for access to females were removed. Since monogamous males barely have to invest in pre-copulatory traits in comparison to polygamous males, they may be unconstrained concerning reproductive tissue investment, a trait that maximizes their fitness even in the absence of male-male competition. Similarly, for females, selection associated to monogamy can unconstrain reproductive tissue investment by removing the need for investment into resistance traits with which to counter male harassment and harm. Thus, our findings point towards the possibility of trade-offs between pre- and post-copulatory sexual selection constraining reproductive tissue investment. Generally, our study underscores the complexities underpinning the evolution of reproductive allocation strategies.



Multidimensional perspective of flower color polymorphism in *Iris pumila*

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POSTER SESSION 2 - P02.138, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Flower colors and color patterns are mediators between plants and pollinators. While differences in flower color between different species (inter-specific polymorphism) are common, color variations within the same species (intra-specific polymorphism) are relatively rare and mostly attributed to balancing selection. The dwarf bearded iris (*Iris pumila* L.) shows a striking variation of flower color in shades of purple and blue, accompanied by yellow and infrequent white flowers. Multiple aspects of *I. pumila* flower color polymorphism were investigated by a combination of spectrophotometric reflectance analysis, biochemical profiling of pigments, and modeling in relevant colorspace. Flower color is therefore interpreted in different ways: as a spectrum of reflected electromagnetic radiation and as distinct composition of relevant pigments – an objective qualification, and as perception of reflectance spectrum by humans and by pollinators – a species-specific qualification. The reflectance spectra were measured using the JAZ spectrophotometer on the perianth tepals, revealing significant differences in brightness, hue and saturation descriptors (colorimetric variables) among color morphs, which were classified into seven clearly distinguishable groups according to human judgment: three shades of purple, three shades of blue, and yellow (the number of color classes in previous studies varied between three and nine). Biochemical assays, based on LC-HRMS/MS analysis identified and quantified flavonoids and anthocyanins responsible for pigment diversity among the color morphs and among the shades of each major color, by comparing them with standard compounds and literature data. For the most part, the composition did not differ between color morphs, and the different colors

were predominantly represented by different concentrations of the pigments studied. Since color is a property of species-specific visual perception, the difference between two colors that is distinguishable to a human may not be distinguishable to a pollinator. The dwarf bearded iris is mainly pollinated by Hymenoptera, most frequently by bumblebees. To evaluate visual perception and visual discrimination by pollinators and humans, quantum catches were estimated, converted into appropriate colorspace and visualized. Flower color was modeled using both the Hexagon model of Chittka and human CIEXYZ and CIELAB colorspace. Results showed that spectral signals are distinctly positioned in hymenopteran colorspace, suggesting potential differentiation in pollinator attraction as measured by distance estimation. The integration of physical, biochemical and perceptual data highlights the complex, multidimensional nature of *I. pumila* flower color and provides a first insight into the potential evolutionary pressures shaping flower color traits.

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Multipartite Parasitic Interactions in the Aquatic Food Web: Modulating Gene Exchange and Carbon Flow

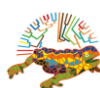
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POSTER SESSION 3 - P03.286, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Parasitism is defined by an imbalanced profit margin where one partner benefits at the expense of the other partner. Detailed studies highlight the importance of parasitic interactions in gene and nutrient flow in the ecosystem and consequently challenge this binary status, suggesting a continuum of interaction dynamics along mutualism and parasitism extremes. Parasites are widespread in aquatic ecosystems with a notable diversity including viruses and zoosporic eukaryotes. These parasites fine-tune their host's dynamics and abundance, and contribute to gene flow in the ecosystem. Their top-down trophic interactions with their host facilitates carbon flow and modulate biogeochemical cycles however its functional basis remains unknown. Majority of our existing knowledge is focused on two-partite parasitic systems that is far from the complex natural interaction web. Studying interactions in a multipartite perspective is challenged by lack of an operational framework. My

research uses multipartite parasitic model systems in culture and multi-omics analyses to understand the functional basis of their interactions and its ecoevolutionary consequences. I apply a framework where in addition to tight co-evolutionary dynamics between pairs in a multipartite system, system-level diffuse co-evolution is also considered. One of our multipartite model systems contains two phages (genome sizes of 166 and 57 Kb) co-infecting a single *Limnohabitans* population. The larger phage represents lysogenic lifestyle and has the opposite codon usage pattern compared to its host and carries all tRNAs needed to compensate for its different codon usage pattern. The transcription profile of the whole pathosystem along the infection cycle showed active transcription of all three constituents. Comparative transcriptomics suggests that the small lytic phage uses additive toxin-antitoxin systems to ensure its persistence in the system.



Multivariate selection and the evolution of *Drosophila* wings

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POSTER SESSION 3 - **P03.301**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

This talk reports two separate experiments aimed at understanding how selection shapes evolution of a complex trait- *Drosophila melanogaster* wings- with implications for understanding both how structural color variation evolves and how multivariate genetic variance is maintained in short and deep time. First, we used a competitive mating assay to assess the degree to which sexual selection acts on male wing interference color independently of wing size and shape. We found that 16% of multivariate wing color, and 17% of corresponding estimated wing thickness, can be explained by wing size and shape. Projection pursuit regression identified three suites of traits – a linear combination of color variables, wing size, and a linear combination of wing shape variables – as the most important predictors of fitness. Analysis of the corresponding selection gradients revealed a combination of strong directional selection on wing size, combined

with multivariate stabilizing selection on wing size and shape, with weak but significant directional selection on wing color. These results (in press at *Evolution*) suggest that sexual selection may act on a complex combination of wing color and morphology in flies.

The aforementioned study found evidence of stabilising selection on at least one suite of wing shape traits, but lacked power to examine nonlinear selection in multiple dimensions. We thus have followed this with a second, larger experiment, where we have obtained measures of fitness and multivariate wing shape from over 2000 flies, in order to estimate a full-rank gamma matrix for all 21 wing shape/size traits. Geometric comparison of this matrix with previously published matrices of standing genetic and mutational variance will provide a test of the hypothesis that stabilizing selection contributes to strikingly-conserved patterns of genetic variance in this complex trait.



Mutations conferring resistance to second-line drug treatments in the Mexican population affected by tuberculosis

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POSTER SESSION 1 - P01.363, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: The development of resistance to drug treatment in *Mycobacterium tuberculosis* (MTB) is related to specific mutations in certain genes that encode drug targets or activating enzymes. The mutations, which confer resistance to second-line drugs, have enhanced our understanding of the spread of multidrug-resistant and extremely drug-resistant strains. Among the drugs used in the second-line treatment of tuberculosis are ciprofloxacin, levofloxacin, ofloxacin, and moxifloxacin. Drug resistance poses a significant threat to global health, underscoring the need for routine surveillance to monitor and control drug-resistant pathogens like MTB. This study aimed to analyze MTB resistance to second-line drugs within the Mexican population.

Materials and Methods: The analysis of available and new genomes of all existing public Mexican isolates was conducted using bioinformatics tools. **Data Collection and Availability.** Raw *Mycobacterium tuberculosis* short Illumina reads were gathered from the following projects: PRJEB30933 (Madrado-Moya et al., 2019), PRJNA824124 (Mejía-Ponce et al., 2022), PRJEB44165 (Rendón-Bautista et al., 2021), and PRJEB75503 (Alvarez-Maya et al., 2025), which can be accessed at the www.ebi.ac.uk/ena repository. Additionally, scaffolds were collected from the project PRJNA751891 (Molina Torres et al., 2022). **Genomic Assembly and Validation.** Raw reads were trimmed and quality-filtered using fastp v0.12.4. Genomic assemblies were performed with SPAdes v3.15.5, utilizing the --careful and --cov-cutoff auto options. **Drug Resistance In Silico Profiling.** Drug resistance profiles, along with MTB lineages and sublineages, were assigned to the strains in silico using TB-profiler v4.3.0. The generated database

was segmented according to the different variables analyzed, using the MS Excel program. **Results:** Lineage 4 was found to be the most prevalent among *Mycobacterium tuberculosis* (MTB), accounting for 93% of the samples. Within this lineage, the most common sublineages included 4.1.2.1 (20%), 4.1.1.3 (19%), sublineage 4.8 (11%), sublineage 4.1.1 (9%), sublineage 4.3.3 (7%), 4.3.4.2 (6%), and 4.4.1.1 (4%). Notably, 75% of the total multidrug-resistant (MDR) isolates were identified within the sublineages 4.1.1.3, 4.3.3, and 4.1.2.1.

In terms of overall frequency among the classified groups, sensitive strains showed the highest occurrence, followed by MDR-TB, then mono-resistant MR-TB and RR-TB, and finally XDR-TB. In this context, the frequency of MDR-TB was higher than that of RR-TB. For fluoroquinolones, the frequencies for the mutations were as follows: gyrA_p.Asp94Tyr (3.5%), gyrA_p.Ala90Val (2.3%), gyrA_p.Asp94Ala (1.7%), gyrB_p.Thr500Asn (0.6%), gyrA_p.Ser91Pro (0.6%), gyrA_p.Gly88Ala (0.6%), and gyrB_p.Thr500Asn (0.6%).

Conclusions: We found that drug resistance varies among different sublineages, and certain single nucleotide polymorphisms (SNPs) appear to be fixed in specific strains. Our study revealed that the mutations responsible for well-known drug resistance in the gyrA and gyrB genes were the most common in fluoroquinolone-resistant isolates. However, many isolates exhibiting pyrazinamide resistance contained previously unidentified mutations that could affect patient management decisions. Therefore, it is essential to improve tools such for sequencing, which involves analyzing large numbers of isolates, to better understand these mutations and predict drug resistance more effectively.



New mutation, gene flow or plasticity? Using genomic variation to test the origins of seasonal allochrony in storm-petrels

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POSTER SESSION 2 - P02.006, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

Background: Allochrony - differences in the time of day, season or year that individuals breed – is widespread in nature, but the roles of new mutations, standing genetic variation, gene flow and phenotypic plasticity (flexibility) in generating and maintaining allochrony are unclear. Storm-petrels are small, pelagic seabirds that breed colonially on offshore islets. Seasonal allochrony occurs in six archipelagos representing two species complexes (*H. castro* and *H. leucorhous*). Previous studies found that birds breeding in different seasons often differ in morphology, diet, vocalizations, and neutral genetic variation, and that allochrony has arisen sympatrically within these archipelagos.

Materials and methods: We investigated the source of seasonal allochrony using a combination of reduced representation and whole genome sequencing of birds sampled throughout the species' breeding ranges. We used a combination of historical

demographic reconstruction, phylogenomics, and co-ancestry analysis to test for founder effects (new mutations), historical or contemporary gene flow, and plasticity in allochronic populations of storm-petrels.

Results and conclusions: Genetic variation was high and similar among populations, arguing against allochrony arising through new mutations. Demographic modeling and phylogenomic reconstructions provided some evidence of historical gene flow or introgression among archipelagos. However, co-ancestry analyses indicated that breeding season may be plastic within several archipelagos, with assortative mating in some locations. These results are consistent with recent evidence from banding at Cabo Verde that birds may switch seasons following failed breeding attempts. The origins of differences in morphology despite plasticity in breeding time remain to be determined.



Non-invasive samples reveal genetic connectivity and fine-scale population structure in western lowland gorillas

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POSTER SESSION 3 - P03.333, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The genus gorilla has two species, the eastern and the western, and each species is divided into two subspecies, three of them labelled as critically endangered and the mountain gorillas as endangered. Genomic analyses of wild endangered populations are benefitting from the advantages of non-invasive samples. This type of samples allows to cover a larger amount of the entire distribution of current wild populations. Here we non-invasively sampled 329 wild gorilla samples from three of the four gorilla subspecies across their present-day distributions, to generate georeferenced genetic data from 37 different sampling sites. We applied the target-capture hybridisation protocol developed for use in chimpanzee faecal samples for the first time to capture chromosome 21 and the exome of wild gorilla faecal samples. In addition, whole genomes were sequenced from 12 gorilla blood samples collected using FTA cards® and 11

gorilla museum specimens. This allowed us to characterise fine-scale population structure in western lowland gorillas at unprecedented resolution. We also reconstruct patterns of past population connectivity between the two western gorilla subspecies, which may have been facilitated by a migration corridor also utilised by chimpanzees. Our detection of geographic barriers, levels of connectivity and isolated populations will be useful for development of conservation strategies for these critically endangered populations. Using the georeferenced genetic dataset we predicted geographic origins for previously-published wild-born gorillas to the subspecies level, and for mountain gorillas and some western lowland gorillas to the population level. These predicted geographic locations provide a first step for future applications to geolocalise trafficked or rescued gorillas.



Nutrient-Driven Forms: Evolved Allometry in *Drosophila* adapted to malnutrition conditions

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POSTER SESSION 3 - **P03.310**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Experimental evolution of *Drosophila melanogaster* on severely nutrient-poor diet for over 300 generations has yielded populations that are notably smaller, develop faster, exploit dietary amino acids with greater efficiency, and accumulate reduced energy reserves compared to unselected controls. Whole-organism RNA sequencing of these “poor-food” lines revealed systematic biases in the expression of gene sets associated with specific anatomical structures, prompting the hypothesis that adaptation under chronic nutritional stress entails not only changes in overall body size but also shifts in the relative growth of distinct organ systems.

To test this idea, we employed high-resolution micro-computed tomography (micro-CT) to non-invasively image and quantify the volumes of multiple organ systems in larvae from both evolved and control populations at two critical developmental milestones: the onset of the third instar and the pre-pupal transition. These 3D reconstructions enabled precise calculation of tissue-specific growth rates across the early third instar and pre-pupal stages. In parallel, larvae sampled at the same stages were subjected to whole-body RNA sequencing to evaluate whether transcriptional biases mirror predicted allometric rearrangements.

Our experimental design addresses two key questions. First, does prolonged adaptation to nutrient limitation preserve proportional scaling across organ systems—or does it promote differential size shifts that reflect functional trade-offs? Second, to what extent do bulk RNA expression profiles capture or exaggerate these anatomical changes? Although we are still finalizing quantitative outcomes, preliminary analyses predict that evolutionary pressures under poor-food conditions will drive detectable allometric shifts during the third-instar growth window, favoring the maintenance of systems critical for rapid development at the expense of others. We further anticipate that these morphological adjustments will correlate with directional biases in whole-organism transcriptomes, offering a mechanistic link between gene expression and adaptive form.

By integrating advanced 3D imaging with developmental transcriptomics, our study pioneers a framework for dissecting how chronic environmental stress reshapes growth trajectories and gene-expression landscapes in real time. This work not only enhances our understanding of the evolutionary plasticity of developmental programs but also demonstrates the power of micro-CT as a tool for revealing hidden dimensions of morphological adaptation.



On the origin of the parthenogenetic, polyploid insect *Saga pedo* (Orthoptera, Tettigoniidae)

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POSTER SESSION 3 - **P03.351**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Saga pedo is a large predatory bush-cricket with a scattered distribution along Europe. It is classified as Vulnerable in the IUCN Red List due to its low population densities and patchy distribution. Two features about *S. pedo* are unusual: it reproduces through obligate parthenogenesis (there are no records of males), and it is the only known polyploid orthopteran. Early cytogenetic studies showed that *S. pedo* has much higher chromosome number than other species from the genus. However, its precise ploidy level remains debated, with reports supporting either tetraploidy ($4n=68$) or pentaploidy ($5n=70$). The evolutionary origin of parthenogenesis and polyploidy in *S. pedo* remains unsolved, although several hypotheses have been put forward.

We performed low-coverage Illumina sequencing on *S. pedo* and five bisexual *Saga* species (*S. gracilis*, *S. campbelli*, *S. rammei*, *S. hellenica*, and *S. rhodiensis*), assembled their mitogenomes as well as nuclear rDNA and snDNA markers to explore the evolutionary relationships of the genus. We also analyzed genome repeat content, and built k-mer-based phylogenies with mixed samples placement. Additionally, we developed cytogenetic markers based on satellite DNA (satDNA) to identify chromosomes from relative bisexual species *S. pedo*, with the goal to identify the putative parental species, using fluorescent in-situ hybridization.

We found that mitogenome phylogenies placed *S. pedo* closest to *S. campbelli*, while nuclear markers (rDNA and U2 snDNA) placed *S. pedo* closest to *S. gracilis*, supporting a hybrid origin. Repeat content and k-mer distance-based analyses suggested that *S. pedo* has genomic contributions from not only *S. gracilis* and *S. campbelli*, but also from *S. hellenica*. FISH using satDNA markers characteristics from different parental species confirmed this chromosomal mosaicism in *S. pedo*, showing two chromosome sets from *S. gracilis*, two sets from *S. campbelli* and one set from *S. hellenica*.

Based on these findings, we propose the origin of *S. pedo* in two successive interspecific hybridization events. A first cross between a *S. gracilis* male and a *S. campbelli* female produced an allotetraploid hybrid (AABB), via hybridization and genome duplication. Subsequently, fertilization of an unreduced egg from this tetraploid female by a haploid sperm from a *S. hellenica* male resulted in an allopolyploid (AABBC) zygote. The resulting odd ploidy number might be related to the obligate parthenogenetic reproduction of *S. pedo*.

Our studies uncover a rare evolutionary pathway that leads to asexual reproduction and polyploidy in animals, and future studies could provide information into the biology and adaptive potential of this threatened species.



One Sea, Different Whales: Genomics Sheds Light on the Puzzling Population of Mediterranean Fin Whales

Desiato E¹, Biello R¹, Iannucci A², Fuselli S¹, Urban Ramirez J⁵, Fossi M³, Panti C³, Mancina A^{1,4}

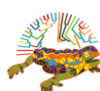
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POSTER SESSION 3 - P03.259, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Whales play a crucial role in marine ecosystems, yet many populations face growing environmental pressures. Among them, the fin whale (*Balaenoptera physalus*) from Mediterranean Sea remains poorly characterized at the genomic level despite its classification as Endangered and decreasing in population size due to habitat degradation, climate change, and human-induced disturbances. Previous studies based on bioacoustic and telemetry data suggest the presence of both resident and migratory subgroups, but the genetic isolation of this population remains uncertain. Here, we sequenced and analysed for the first time whole genomes of the Mediterranean fin whale to provide a comprehensive genomic characterization of this population, assessing its genomic variability, genetic load, population structure and potential for adaptation to environmental stressors. By comparing genomic data from Mediterranean fin whales with populations from the North Atlantic, North Pacific, and Sea of Cortez, we aim to determine the degree of genetic isolation and

contextualize the Mediterranean population within a broader evolutionary and conservation framework. Our results showed that while Mediterranean fin whales form a distinct genetic cluster, they are not completely isolated from North Atlantic populations, with gene flow persisting at low levels. Additionally, we identified a complex substructure within the Mediterranean, supporting the existence of a resident subpopulation. Although this population preserves moderate levels of genomic diversity and adaptive potential, it remains vulnerable to genomic erosion due to continued demographic decline, limited connectivity, and increasing environmental stress. These findings underscore the urgency of targeted conservation actions and long-term genetic monitoring, especially as climate change accelerates and introduces increasingly unpredictable selective pressures that may exacerbate the risk of genomic erosion and threaten population viability.



One species, many shells: Heritable habitat-associated divergence in the sole lymnaeid snail species of southern Patagonia

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POSTER SESSION 1 - **P01.043**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Variation within species is widespread across the living world, arising from genetic differences, environmental influences, or both. Regardless of its origin, intraspecific variation not only shapes ecological interactions but also poses major challenges for systematics and taxonomy. Freshwater snails, key vectors of parasitic trematodes affecting human and livestock health, exemplify this problem: their high shell shape variability complicates taxonomic identification and challenges the accurate delineation of species distributions. Addressing these issues requires integrative approaches combining biogeography, morphometric analyses, and the evaluation of phenotypic plasticity.

Here, we focus on *Pectinidens diaphanus*, a lymnaeid snail and potential vector of fascioliasis, a globally distributed zoonotic disease. Historically reported from various regions across the Americas, many records were based solely on shell morphology and are now considered likely misidentifications due to morphological convergence with other lymnaeids.

To date, *P. diaphanus* has been reliably confirmed only at two localities in Tierra del Fuego, at the southernmost tip of Patagonia. Despite its potential epidemiological importance, this species remains poorly characterized, particularly regarding its true distribution and patterns of morphological variation across environmental gradients. In this study, we reassessed its geographic range and investigated habitat-associated shell shape divergence across southern Patagonia.

Between 2018 and 2023, we conducted field surveys at 134 sites across southern Argentina and Chile, sampling lymnaeid snails from both temporary and permanent habitats. A subset of individuals was preserved for anatomical and genetic analyses to confirm species identity. To investigate habitat-associated morphological variation, we selected five representative populations spanning a gradient of habitat permanence—from stable environments (lake, river) to temporary ones (stream, pond). We

performed geometric morphometric analyses on 107 standardized shell images. We also measured the perimeter and surface area of the prostate and penial complex, structures commonly used in lymnaeid taxonomy. To test whether shell shape divergence was driven by plasticity or heritable variation, we established laboratory populations from wild snails collected from lakes and ponds. Individuals were reared under standardized laboratory conditions (16 °C, 16:8 h light:dark cycle, food ad libitum) for two generations (F2), and the same morphometric analyses was applied to F2 snails.

Of the surveyed sites, 42 were positive for lymnaeid snails. Anatomical and genetic analyses confirmed that all specimens belonged to *P. diaphanus*. Contrary to previous reports, the species occupied a much broader region of southern Patagonia, with no evidence of other lymnaeid species co-occurring at any site. Shell shape varied consistently with habitat type: individuals from stable, permanent environments exhibited more globose shells, whereas those from temporary habitats developed more elongated forms. In contrast, internal reproductive morphology showed no significant differences across habitats. Notably, F2 individuals retained the shell shape patterns associated with their habitat of origin, mirroring wild populations and indicating a heritable component to shell shape divergence.

Our findings represent the most comprehensive spatial and morphological assessment of *P. diaphanus* to date, extending its confirmed range across southern Patagonia and demonstrating its ecological versatility. The persistence of shell shape differences across generations points to underlying genetic differentiation rather than environmentally induced plasticity. Altogether, these results underscore how environmental heterogeneity can leave a lasting evolutionary imprint on morphology, with major implications for species identification, local adaptation, and the evolutionary ecology of host–parasite systems.



Origin, evolution and biogeography of Atlantic Forest Nymphalidae (Lepidoptera)

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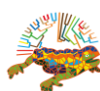
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POSTER SESSION 3 - P03.269, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Using phylogenetic and biogeographic analyses, we investigated the key macroevolutionary processes that may have shaped the extant diversity in the Atlantic Forest. Today, with only 11.7% of its original vegetation remaining and facing severe threats from human activities, this domain is considered one of the most critical biodiversity hotspots on Earth. Throughout its area, congruent patterns of species richness, diversity and endemism are shared between taxa, but the evolutionary processes that led to them are still unknown. Butterflies, with their well-documented data, serve as an ideal model for investigating these evolutionary dynamics. Here we investigate the macroevolutionary history of Atlantic Forest Nymphalidae by sequencing missing species in time-calibrated phylogenies, compiling published distribution data and applying macroevolutionary tools to delineate the speciation and lineage dispersal dynamics. We test whether the forest's nymphalid diversity arises from in situ speciation, Neotropical dispersal, or both, and their trends through time. In addition, for its higher concentration of species richness, we explore the potential relevance of altitude in the diversification history of the domain. We included 3600 nymphalid samples for the phylogeny inference –including genetic data from the two major up-to-date studies

(Chazot et al. 2021, Kawahara et al. 2023) and new whole genome sequencing– and 1460 unique species for the biogeographical analyses. Our results highlight the Andes as the main source of Nymphalidae diversity for both the Atlantic Forest and Neotropical regions as a whole, showing the highest number of dispersal events out of it, highlighting the role of connectivity between these two regions during the Miocene. The Amazon was recovered as the second source of Atlantic Forest diversity, gradually increasing since 15 million years ago, supporting the existence of forest corridors throughout the Dry Diagonal during this period. On the other hand, the speciation rate within the region has remained relatively constant, but with different dynamics in the southern and northern regions of the domain. Overall, we showed that the Atlantic Forest nymphalid diversity resulted primarily from a constant dispersal over time until about 15 million years ago (Mya), when it accelerated, coupled with a steady increase in speciation until approximately 20 million years ago. By targeting butterflies—rarely used in Atlantic Forest biogeography, which centers on plants and vertebrates—we revisited old hypotheses and expanded our understanding of the region's evolutionary history.



Origins of androgenetic lineages and sperm competition in the clam genus *Corbicula*

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POSTER SESSION 3 - With Aperitif, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Androgenesis is an uncommon reproductive mode where the offspring inherit exclusively the paternal nuclear genome, the maternal nuclear chromosomes are either absent or extruded from the oocyte during fertilization. Despite its rarity, this phenomenon is prevalent within the bivalve genus *Corbicula*, which includes both sexual and androgenetic lineages. Sexual *Corbicula* species are dioecious, restricted to the native range (Asia, Africa and Australia), and their males produce reduced, monoflagellate sperm. In contrast, androgenetic lineages are hermaphroditic, they're present in both the native and the invaded range, and they're characterized by the production of unreduced, biflagellate sperm. In these androgenetic lineages, fertilization is accompanied by the extrusion of the entire maternal chromosome set as two simultaneous polar bodies.

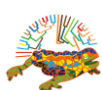
Previous molecular analyses (Vastrade et al., 2022) demonstrated extensive allele sharing between three invasive androgenetic lineages and the sexual dioecious species *Corbicula sandai*, which is restricted to Lake Biwa (Japan). More recently, Etoundi et al. (2024) observed that males of *C. sandai* produce simultaneously monoflagellate reduced and biflagellate unreduced sperm in *C. sandai*, raising questions about the evolutionary origin of androgenesis in this genus. Additionally, the study conducted by Vastrade et al 2022 also revealed a second allele sharing between the fourth androgenetic invasive lineage, namely "form C/S" (Europe and America), and a population of *Corbicula fluminalis africana* from Mooi river (South Africa), whose reproductive traits remain unknown.

My PhD project addresses two main questions: (i) Did androgenetic *Corbicula* lineages originate independently from sexual ancestors in Asia and Africa? (ii) Does the production of biflagellate, unreduced sperm confer an a fitness advantage when compared to the production of monoflagellate reduced sperm?

Over the initial two years of my PhD, we established spawning protocols and conducted initial observations of sperm morphology across native and invasive *Corbicula* populations. Invasive populations from the Vidourle River (France) and the Waal River (Netherlands) exhibited gametic characteristics consistent with androgenesis, notably biflagellate sperm with a mean head length of 18.7 μm . In some individuals, both sperm and oocytes were observed, with meiotic spindles oriented parallel to the oocyte cortex, indicative of simultaneous polar body extrusion.

In contrast, individuals from Lake Biwa and adjacent riverine populations (Hikone region) predominantly produced sperm with relatively small (5–10 μm head length), narrow heads and poorly distinguishable flagella under 600x magnification. Notably, some individuals from rivers surrounding Lake Biwa produced also a small proportion of biflagellate spermatozoa (~15 μm head length), morphologically similar to those observed in invasive lineages. While several individuals from surrounding river populations released both sperm and oocytes, individuals from Lake Biwa released sperm exclusively, consistent with their dioecious classification as *C. sandai*.

These preliminary findings could support the dioecy in populations from lake Biwa, while also revealing unexpected sperm diversity in the river populations. To further investigate these observations, additional analyses are required, including genome size estimation in both sperm and somatic cells to assess sperm reduction or non-reduction, histological examination of gonadal tissues to determine the sexual phenotype of individuals (hermaphrodites or dioecious), and genetic identification to clarify the taxonomic and phylogenetic status of the sampled populations.



Parental age effects on offspring telomere length across vertebrates: a meta-analysis

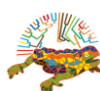
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¹University Of Groingen

POSTER SESSION 1 - with Aperitif, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Telomeres shorten with advancing age in numerous species, and shorter telomeres are linked to increased mortality risk. While parental age at conception can influence offspring telomere length, the magnitude and direction of this effect differ across studies, species, and parental sexes. A significant knowledge gap therefore exists in understanding how parental age influences offspring telomere length across vertebrate species. To address this knowledge gap, we conducted a systematic review and meta-analysis to examine the effects of paternal and maternal age at conception on offspring telomere length, incorporating 99 effect sizes from 30 human studies and 49 effect sizes from 12 non-human vertebrates. Within the human studies, there was a positive overall parental age effect on offspring telomere length, while no effect was found in the non-human vertebrate studies after adjusting for study, estimate, and

phylogenetic effects. There was considerable heterogeneity in both human and non-human studies. In human studies, the heterogeneity was mainly attributed to between-study variance, and in the non-human studies, phylogeny explained a major proportion of heterogeneity. Parental age effect estimates were correlated with the laboratory methods used for measuring telomere length in all studies. In the human studies, parental sex, offspring sex, their interactions, and the tissue type used for telomere extraction affected the parental age effect estimates. In non-human vertebrates, the parental age effect estimates were more positive when the parents' identity was controlled in the study. We recommend that future research be conducted on a broader range of taxa, test for within-parent effects, and follow standardized reporting practices to enhance data comparability.



Parental impact on genetic inheritance: patterns of accumulating mutations across generations in *Macaca fascicularis*

Park H^{1,2}, Cho H¹, Choe S¹, Ko M^{1,2}, Lee Y^{1,2}, Park S¹, Kim Y¹, Huh J^{1,2}

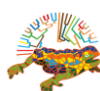
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POSTER SESSION 3 - P03.275, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Mutations are alterations in the genetic sequence that typically arise due to errors during DNA replication, often induced by environmental factors such as radiation or chemicals. These changes can occur in somatic as well as germ cells and tend to accumulate incrementally over the course of an individual's life, thereby contributing to the biological processes associated with aging. Of particular significance are germline mutations, which arise in reproductive cells and can be transmitted to offspring, and de novo mutations, which emerge spontaneously in the subsequent generation. The analysis of such mutations provides critical insight into hereditary disorders and facilitates the investigation of genetic abnormalities within familial lineages. In the present study, we performed whole genome re-sequencing (WGRS) using

whole blood (30x coverage) and semen (50x coverage) to analyze mutation rates and patterns in crab-eating monkeys (*Macaca fascicularis*). We selected four parent-offspring trios (a total 16 monkeys; 10 males, 6 females) representing various life stages to collect genomics data across age points. As a result of 4 years research, WGRS data of monkey blood and sperm cells was built up, and based on that, diverse pipelines were devised to analyze mutations accumulated annually. We performed a longitudinal quantitative analysis to assess the accumulation of mutations in the genome over a four-year interval, specifically evaluating de novo mutations in offspring and mutational profiles in male monkey spermatozoa. Additionally, we performed a quantitative pattern analysis with respect to annual variation and blood relationships.



Philopatry, Dispersal, and Distance: Disentangling Genetic Structure in a Highly Mobile Terrestrial Bird

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POSTER SESSION 3 - **P01.137**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Geographic distance and physical barriers are well-established drivers of genetic differentiation, but behavioral traits and habitat preferences can also restrict gene flow. This is particularly relevant in species with high dispersal capacity, where demographic panmixia is often expected, yet ecological traits may promote population differentiation. Patterns of genetic variability have been examined in highly mobile marine bird species, where philopatry restricts gene flow. However, this remains poorly understood in terrestrial birds, where geographic features may exert a stronger influence on population structure. Highly mobile species therefore provide a valuable framework for investigating how ecological and spatial factors interact to shape genetic differentiation.

We explore this dynamic in the oilbird (*Steatornis caripensis*), a widely distributed non-migrant Neotropical bird with high mobility and strong philopatric behavior.

Using whole-genome resequencing, we will analyze individuals from seven populations across Colombia (4), Venezuela (1), and Peru (2), employing population genetic and demographic approaches to assess patterns of genetic variability and structure. We aim to differentiate between three patterns: (1) low genetic differentiation due to extensive gene flow attributed to high dispersal capabilities; (2) population structure driven by behavioral factors such as philopatry, independent of geographic features; and (3) structure associated with geographic distance or barriers limiting gene flow. These patterns are not mutually exclusive and may act in combination to shape population structure. This study contributes to a deeper understanding of how genetic structure arises in highly mobile organisms and offers valuable insights into the evolutionary dynamics and conservation strategies for *S. caripensis* and similar species.



Plasticity in rapidly changing nightscapes: Effects of Artificial Light at Night on color dynamism in chameleons from Madagascar

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POSTER SESSION 3 - **P03.323**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Increasing urbanisation is associated with expanding levels of Artificial Light At Night (ALAN) worldwide. ALAN leads to changes in animal fitness and behaviour, such as impacts on migration routes leading to higher mortality rates, and activity extension and use of more visual cues for hunting with further effects on predator-prey interactions. Hence, ALAN represents a novel selective pressure on biodiversity. Despite this, we lack knowledge of how ALAN and its cascading effects through species interactions may select morphological traits. ALAN can significantly change the conspicuousness of predator and prey color to each other, hence altering predator-prey dynamics. Because of this, we expect color to be under strong selection under ALAN. However, highly plastic morphological traits, such as dynamic color change, may evade such selective pressures. We explore the effects of ALAN on dynamic color change in chameleons during periods of rest when they are highly vulnerable to predation. Since they may be able to avoid predation risk by modifying behaviour or physiology, we further study how their activity or alertness at night is impacted by ALAN. We do so in urban and rural populations, and across days, to further study if animals can acclimate to ALAN.

Methods and Materials: We conducted ex-situ experiments in Madagascar on *Furcifer lateralis*, a chameleon species found across urban and rural areas of the island. Chameleons exhibit circadian changes in color, where they get lighter at night during times of rest. However, color change in chameleons is also known to be photosensitive and hence we expected

individuals to be darker under ALAN treatments. Individuals from urban and rural sites were exposed to either moonlight conditions or white- or amber-colored LEDs at around seven lux for eight nights followed by initial acclimation to moonlight conditions, and then again to moonlight at the end of the experiment. We recorded their color at night using a spectroradiometer. Additionally, we recorded their activity or alertness by recording movement and eye opening at two-hour intervals throughout the night.

Results: Individuals resting under either of the ALAN treatments were significantly darker than those under moonlight. However, all individuals were able to recover under moonlight irrespective of the treatment they underwent. These effects are less pronounced for urban populations compared to rural ones, suggesting potential adaptation towards artificial light at night in the cities. We also find that longer exposure to ALAN leads to dampening of the effects, indicating potential acclimation. The effects of ALAN are also similar on activity or sleep disturbance where individuals resting under ALAN are highly disturbed and easier to wake up.

Conclusions: Our study highlights the impacts of ALAN on species resting at night. Specifically, even plasticity in morphological traits may not allow animals to cope with rapid changes in their environment. With chameleons of Madagascar being endemic to the island, we hope to use the results from this study to inform future development and policies in urban environments.



Plugging into Innovation: Spatial Transcriptomics of Tissue Organization and Gene Expression in the Ricefish Plug

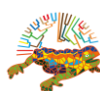
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POSTER SESSION 1 - P01.345, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The so-called "plug" is a transient, unique structure only found in Sulawesi ricefish (Belontiiformes: Adrianichthyidae). It develops within the female gonoduct after spawning and anchors egg-attaching filaments for external brooding. Previous research on the transcriptome of the plug, which shows inflammatory signals and histological sections containing multinucleated giant cells (fused macrophages), suggests that its formation is triggered by a modified inflammatory response. To better understand this process, we profiled the spatial gene expression landscape of the female reproductive system of *Oryzias latipes* at four different time points. We found that specific gene

expression clusters correspond to the morphological tissue organization of the reproductive system, with some being specific to later time points of brooding. Key marker genes for immune cells (e.g. *il1b*, *tgfb1*, *SMS*, *VMP1* or *MRC1*) are specifically expressed in the developing plug and the surrounding tissue, with spatial locations of highest expression intensities differing between time points. The present findings highlight the complex dynamics of pelvic brooding and demonstrate that inflammation, which is typically associated with undesirable outcomes such as granuloma formation, can be co-opted to obtain novel structural components.



PopLife: Population Genomics across the Tree of Life

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POSTER SESSION 3 - P03.282, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The rapid advancement of sequencing technologies is generating an unprecedented volume of population genomic data across an increasing number of species. This data holds precious information about the evolutionary history of life on our planet. To facilitate its interpretation, we present PopLife, a long-term initiative and open-access online platform for population genomics that enables the analysis and visualization of genetic variation within and between populations across the Tree of Life.

PopLife integrates standard population genetics methodologies with state-of-the-art artificial intelligence approaches to extract knowledge from large-scale, heterogeneous genomic datasets. It features a high-performance pipeline for computing key population statistics, such as variation metrics, linkage disequilibrium parameters, and neutrality tests, and a JBrowse-based genome browser for intuitive visualization, on-the-fly analyses, and data retrieval.

By integrating diverse omics datasets, including genome-wide resequencing, Hi-C,

ChIP-seq, and ATAC-seq data across species, PopLife enables the joint analysis of genetic, structural, and regulatory genomic layers. Its AI-powered framework facilitates the discovery of complex patterns and associations, such as correlations between chromatin features and the distribution, density, and types of genetic variants. This intersection of chromatin biology with population genomics offers a powerful approach to uncover regulatory and structural constraints shaping genome evolution, shedding light on how 3D genome architecture may influence recombination, genetic variation, and adaptation, and addressing other fundamental, yet underexplored, questions in evolutionary genomics.

With its versatile analytical capabilities and emphasis on interoperability, PopLife aims to become an international reference for population genomics, supporting research across evolutionary biology, functional genomics, conservation genetics, genetic improvement, and beyond.



Population genomics of coastal dune building species European marram grass (*Calamagrostis arenaria*)

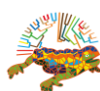
Baker E¹, Curran E¹, Dunning L, Bailes E

¹University Of Sheffield

POSTER SESSION 3 - **P03.305**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Coastal dune systems provide vital natural protection for shorelines, acting as physical buffers against the increasing threats of sea-level rise and coastal storm events. Central to the formation and long-term stability of these dunes are dune grasses, which trap and stabilise sediment, shaping dune morphology through biogeomorphic feedbacks. Despite their ecological importance, the genetic diversity and adaptive potential of these species remains under explored. In this study, we investigate the population genetic structure of European marram grass (*Calamagrostis arenaria*), a dominant pioneer species in dune systems across its native range. We generated nuclear SNP data using double-digest

restriction-site associated DNA sequencing (ddRAD) for 288 individuals sampled from coastal dune systems in the United Kingdom, Sweden, Denmark, Belgium, the Netherlands, and Portugal. Population genomic analyses, including principal component analysis (PCA), revealed clear genetic structuring with distinct clustering by country of origin. These patterns suggest limited gene flow among populations and indicate that regional environmental pressures may be driving local adaptation. Ongoing pairwise F_{st} analyses will quantify population divergence and provide further insight into evolutionary processes shaping variation in the species.



Post-copulatory sexual selection intensity is associated with vagina length but not sperm storage traits in Galliformes

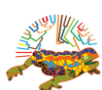
Assersohn K¹, Hemmings N¹

¹The University Of Sheffield

POSTER SESSION 1 - P01.165, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Post-copulatory sexual selection is a powerful evolutionary force capable of driving rapid diversification and shaping some of the most remarkable traits in the animal kingdom. The female reproductive tract provides the arena for post-copulatory processes, yet most research has focused on sperm and male traits, leaving female-mediated mechanisms comparatively understudied – a common occurrence across the field of sexual selection. We address this gap using a comparative framework across 26 different species of Galliformes birds, to investigate relationships between female reproductive tract morphology, sperm competition, and sperm traits. We reveal that vagina length is

positively associated with sexual selection intensity across species, suggesting that the vagina plays an important and currently overlooked role in generating post-copulatory sexual selection. However, contrary to predictions, we find no association between the remarkable morphological diversity in female sperm storage tubule morphology and either sexual selection intensity or sperm traits. Our study raises several important outstanding questions for future research across reproductive biology, fertility, and sexual selection, and provides an important contribution to the growing body of evidence that female genital tract morphology covaries with male genital traits across taxa.



Postglacial divergence and local adaptation in the wood-decay fungus *Trichaptum abietinum*

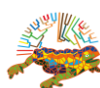
Helleland K¹, Andersen A, Lü D, Kauserud H, Skrede I

¹University Of Oslo

POSTER SESSION 3 - **P03.254**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Fungi are integral to terrestrial ecosystems, notably as primary decomposers with wide-ranging effects on global carbon and nutrient cycling. Given the importance of boreal forests as significant carbon reservoirs and the accelerating climate change, it's crucial to understand the adaptability of wood-decaying fungi in these ecosystems. The focal species *Trichaptum abietinum* is an abundant wood-decomposer throughout the northern hemisphere and is present across a broad range of climatic zones. Recent analyses have identified distinct genetic lineages of *T. abietinum* on either side of the mountains in eastern and western Norway, likely originating from different glacial refugia. Intriguingly, limited admixture has been detected between these lineages despite their ability to mate in vitro. In this study, we explore whether *T. abietinum* has adapted to local climates and identify environmental drivers of adaptation. For 48 individuals spanning a broad climatic gradient across Norway, hyphal extension was measured in vitro on petri dishes across different temperatures and water potentials, along with decay ability on wood blocks of

the main substrates *Pinus sylvestris* and *Picea abies*. Statistical analyses revealed adaptations to local climates, with precipitation and temperature playing key roles in explaining individuals' growth responses. Whole genome sequencing of the individuals identified the distinct genetic lineages and an admixed lineage highlighting a hybrid zone. Linking the genetic information with the phenotypic growth revealed that the pattern of adaptation to environmental stressors is different for the three genetic lineages. These results suggest that local adaptation to climatic conditions may contribute to the maintenance of lineage separation despite potential for interbreeding. These patterns likely reflect historical processes linked to post-glacial recolonization from separate refugia, combined with ongoing adaptation to local environments. Together, our findings highlight how historical migration events and contemporary selective pressures interact to shape genetic structure and may ultimately drive speciation processes in saprotrophic fungi.



Potential Application of Disease Associated SNPs for Forensics: A study on the Kuwaiti population

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POSTER SESSION 1 - P01.359, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

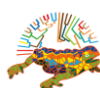
Background: Selecting single nucleotide polymorphisms (SNPs) with high discriminatory power for forensic applications requires that such markers be highly informative within the target population. In Kuwait, the slow progress of the Kuwaiti Human Genome Project presents a challenge in identifying suitable population-specific forensic SNP markers. This study aimed to explore an alternative source for ethnic-specific SNPs by utilizing markers associated with common population diseases.

Methodology (Materials and Methods): SNP data, previously generated by Sanger sequencing, at eight genes. Specifically, genes which have investigated for their effect on the prevalence of obesity and metabolic disorder in Kuwait (APOA I, APOC II, APOA V, APOAIV, APOB, APOC III, APOE & LPL) involved in lipid metabolism were obtained from the Kuwaiti Genetics Database curated by Prof. Suzzane Al-Bustan. An initial dataset of 323 SNPs was subjected to a two-step filtration process to identify markers relevant

for forensic identification. Only bi-allelic SNPs with a minor allele frequency (MAF) ≥ 0.05 and in Hardy-Weinberg Equilibrium (HWE; $P > 0.05$) were retained for further analysis. Haplotype blocks were also constructed from the selected SNPs. Both individual SNPs and their corresponding haplotypes were evaluated for their power of discrimination (PD).

Results: 85 SNPs were determined to be forensically relevant after filtration. The PD of individual SNPs was found to be higher than that of their haplotypes, although still lower than the PD typically associated with short tandem repeats (STRs). Nevertheless, the PD values of the selected SNPs were sufficient for forensic identification within the Kuwaiti population.

Conclusion: These findings suggest that disease-associated SNPs can serve as viable forensic identity markers in Kuwait. Moreover, they offer a supplementary resource for population-specific genetic data, contributing to both forensic investigations and the broader goals of the Kuwaiti Human Genome Project.



Quantity over quality? Parental senescence effects in an annual killifish

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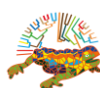
¹Institute Of Vertebrate Biology, Czech Academy Of Sciences, ²CEITEC, Masaryk University

*POSTER SESSION 1 - **P01.167**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM*

Senescence is age-related decline in survival and reproduction. It occurs across multiple life functions and traits but not synchronously. Evolutionary theory of senescence predicts, for example, that decline in parental effects and fecundity may follow divergent trajectories. Annual killifish is a group of short-lived fish from temporary habitats with accelerated life history. They survive pool desiccation in sediment egg banks and hatch at once after the drought period when the pool is flooded. We aimed to test the effect of ageing on quantity, viability of the offspring and related parental effects in a wild-derived strain of turquoise killifish (*Nothobranchius furzeri*, Cyprinodontiformes). We monitored senescence in parental fish at both performance and cellular level in a longitudinal captive study using factorial breeding design. Ultimately, we wanted to

test if older parents also produce biologically “older” offspring. Such offspring would be inferior in the stiff competition when natural pools are flooded.

We recorded large individual variation in parental age-related decline in fecundity and fertilization rate. Offspring of older parents performed the worst compared to offspring of younger parents was the drought survival. Offspring that survived this critical stage and successfully hatched grew at a similar rate irrespective of parental age. It may thus be that the annual killifish parents trade off the quality of their offspring to increase their number. This may allow annual killifish to hedge their bets in the unpredictable environment of ephemeral pools. We currently investigate whether telomere length and DNA methylation patterns correspond with our observation at the population and individual levels.



Recent Positive Selection in a Wild Rodent: Investigating the Genomic Basis of Adaptation

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POSTER SESSION 3 - **P03.268**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Understanding the genomic basis of adaptation in natural populations remains a fundamental goal in evolutionary biology. Positive selection, a key driver of evolutionary change, may target different genomic regions (protein-coding sequences or regulatory elements) depending on the traits under selection and the environmental pressures shaping them. Moreover, certain categories of genes—for example, those involved in host-pathogen interactions—are often expected to be particularly important targets of positive selection.

While protein-coding sequences have traditionally dominated discussions of adaptive evolution, recent studies suggest that non-coding and regulatory regions may play equally pivotal roles. Yet, few genome-wide studies in wild mammals have explicitly quantified the relative contributions of these genomic compartments or linked signatures of selection to functional gene categories.

In this study, we used whole-genome resequencing data to investigate the genomic landscape of recent positive selection in a natural population of the bank vole (*Myodes glareolus*), a widespread Palearctic rodent. By integrating the signature of positive selection as identified by Sweepfinder2 with population genetic statistics (including nucleotide diversity, Tajima's D, and Fay and Wu's H) in sliding

windows across the whole genome, we have identified loci exhibiting strong deviations from neutral expectations, consistent with recent hard sweeps. These candidate regions were then classified according to their genomic context and further annotated to assess functional enrichment via Gene Ontology and pathway analyses.

Among our preliminary findings, several immune-related genes appear to show signals of selection, which would align with the hypothesis that host-pathogen interactions may act as important evolutionary forces. Interestingly, we also observed that several genes under selection are involved in DNA repair processes, with many having known associations with cancer in humans.

Our findings may provide insights into the architecture of selection, for example, whether candidate regions tend to fall within or outside genes, raising the possibility that adaptive changes might not be limited to coding DNA.

These results will have broader implications for understanding pathogen-mediated selection and adaptive evolution in general. It also highlights the value of integrating functional annotation with population genomic scans to move from detecting selection to interpreting its evolutionary significance.



Reconstructing the morphological and chemical evolution of the prothoracic repellent glands in stick and leaf insects (Phasmatodea)

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POSTER SESSION 3 - **P03.324**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Phasmatodea are well known for their astonishing ability to disguise themselves as twigs, leaves, or bark, and are therefore commonly known as stick and leaf insects. In addition to this beneficial defensive strategy, many phasmatodean species use prothoracic repellent glands to spray defensive chemicals when disturbed by predators or parasites. We investigate the anatomy and the chemical substances for a global and representative sampling of stick and leaf insects by use of μ CT scans and gas chromatography/mass spectrometry (GC/MS). The glands are located pairwise inside the thorax. In some species developed as small sac-like structures at the tip of the prothorax, in other species as massive tubes proceeding through the whole thorax. Thus, the size and structure can notably differ and several taxa independently evolved huge

glands in the new world and old world Phasmatodea. Regardless of the gland size, numerous stick and leaf insects use the same chemical compound as main ingredient of their repellent secretion, a bicyclic monoterpene, which is highly irritating for mucus membranes and the eyes, likewise functions as a repellent against parasites. We could identify the ground pattern molecule of the defensive secretion of the Phasmatodea, which seems to be unchanged in several taxa since 150 million years. This molecule is often modified and an isomer is used in the Euphasmatodea. However, there are many species that evolved completely new substances in their repellent gland secretion, especially in the Necrosiinae and the Malagasy clade.



RECUR: Identifying recurrent amino acid substitutions from multiple sequence alignments

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POSTER SESSION 1 - **P01.081**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Identifying recurrent changes in biological sequences is important to multiple aspects of biological research - from understanding the molecular basis of convergent phenotypes, to pinpointing the causative sequence changes that give rise to antibiotic resistance and disease. Here, we present RECUR, a method for identifying recurrent substitutions that have occurred in a protein or codon multiple sequence alignment. We demonstrate the utility and performance characteristics of RECUR on a data set of surface glycoprotein (S) protein sequences from SARS-CoV-2.

Materials and methods: RECUR takes a multiple sequence alignment as input and identifies all recurrent sequence substitutions present within the evolutionary history of that alignment and their associated statistics. Specifically, a phylogenetic tree is constructed from the input multiple sequence alignment and ancestral sequences are inferred for all internal nodes of the phylogenetic tree. All amino acid substitutions are then identified by assessing changes in the protein sequence along every branch in the phylogeny. Simulated multiple sequence alignments are generated using the inferred ancestral sequence, phylogenetic tree, and the best-

fitting model of sequence evolution. These simulations are then used to identify recurrent substitutions that occur more frequently than expected, given the number of sequences, their evolutionary relationships, and the chosen model of sequence evolution. A standalone implementation of the algorithm is available under the GPLv3 licence at <https://github.com/OrthoFinder/RECUR>.

Results and conclusion: We show that RECUR is a comprehensive tool for identifying amino acid substitutions from multiple sequence alignments that is fast and scalable to thousands of sequences. Through analysis of 123,126 S protein sequences from SARS-CoV-2, we identified widespread recurrent evolution throughout the protein. In silico modelling showed that recurrent substitutions have primarily acted to stabilise the trimeric interface of the S protein homotrimer, but had no consistent effect at the hACE2 receptor interface, indicating that these sites are not evolving solely to increase hACE2 binding affinity but also evade the host immune response. We anticipate that RECUR will serve as a valuable resource for understanding the molecular basis of convergent phenotypes as well as generating hypotheses and guiding experimental testing of protein function.



Repeated sex chromosome turnovers in African annual killifishes

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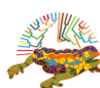
POSTER SESSION 3 - **P03.277**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Sex chromosomes have evolved many times across eukaryotes. Theory predicts that once a sex determining (SD) locus arises, it drives progressive suppression of recombination resulting in convergent molecular differentiation. However, evidence from taxa like teleost fishes, representing over half of vertebrate species with unmatched diversity in SD systems, challenges this model. Sex chromosomes are difficult to detect in teleosts as they experience frequent turnovers resetting the differentiation process. The *Nothobranchius* killifishes offer an excellent model for studying sex chromosome turnovers. While *N. furzeri* and *N. kadleci* share the XY system, derived X1X2Y systems were reported in six other species across the genus.

Materials and methods: We combined molecular cytogenetic and genomic analyses to test whether the *Nothobranchius* X1X2Y sex chromosome systems evolved independently. We performed physical mapping of five of these multiple sex chromosome systems using fluorescence in situ hybridization with bacterial artificial chromosomes bearing orthologues of *Dmrt1* and members of the TGF- β signaling pathway, namely *Amh*, *Amhr2*, *Gsdf* and *Gdf6*, repeatedly co-opted as master sex determining genes in fishes. We further sequenced genomes of *Nothobranchius guentheri* and *Fundulosoma thierryi*, together with sex-specific DNA pools, to identify their putative SD genes and assess levels of sex chromosome differentiation.

Results: We revealed at least four sex chromosome turnovers in *Nothobranchius* killifishes. The *Gdf6* gene was sex-linked in the outgroup *F. thierryi*, indicating its co-option as an SD locus in *F. thierryi* and *N. furzeri*/*N. kadleci*. Additionally, the X1X2Y systems of *N. guentheri*, *N. lourensi* (both Coastal clade), and *N. brienii* (Kalahari clade) shared a potential SD region, which fused with different chromosomes. Indeed, we uncovered two sex-linked evolutionary strata of distinct age in *N. guentheri*. The *Amhr2* gene was sex-linked but located in the younger stratum and is hence unlikely to be the ancestral SD gene in this lineage. Other sex-related genes became sex-linked through the sex chromosome-autosome fusions in *N. guentheri* and *F. thierryi*, possibly facilitating their fixation. None of the usual suspects was linked to multiple sex chromosomes of *N. ditte*.

Conclusions: Our cytogenomic study uncovered dynamic evolution of sex chromosomes across the speciose *Nothobranchius* genus and factors influencing the emergence of new sex-determining systems. Recurrent turnovers repeated co-opted TGF- β -pathway genes and involved recombination cold spots such as centromeric regions and fusion breakpoints, that may promote recombination suppression and subsequent differentiation of sex-determining regions. We demonstrate combined cytogenetic and genomic data can address knowledge gaps in our understanding sex chromosome evolution.



Reproductive success of dispersers depends on the population of origin in Atlantic salmon

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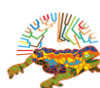
²Université de Pau et des Pays de l'Adour, UPPA, INRAE, ECOBIOP, Saint-Pée-sur-Nivelle, France,

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POSTER SESSION 3 - P03.250, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Atlantic salmon (*Salmo salar*) is an iconic species of great socio-economic value, which is also known for its philopatric behaviour called 'homing'. After spending one to several years in freshwater, juveniles migrate to marine areas for growth, remaining there for one to several years before returning as adults to their natal river to reproduce. However, some individuals can disperse to nearby or more distant rivers, leading to a metapopulation functioning. Theory predicts that dispersing individuals may have a lower fitness than local individuals, but this prediction has been rarely investigated in Atlantic salmon. The objectives of this study were to: i) identify the dispersers in a focal population (Nivelle River, France), and ii) compare their reproductive success with local individuals. We combined pedigree and population

genetic approaches using 4331 individuals genotyped with 14 microsatellites and/or 164 SNPs over a sixteen years period. Population genetic assignment tests coupled with pedigree reconstruction revealed a high dispersal rate of 19.5% into the Nivelle population. Dispersal was male-biased and higher in single-sea-winter salmon than multi-sea-winter fish. On average, reproductive success was 15% lower for dispersing individuals than local ones. However, after accounting for sex and sea age, dispersers from some populations had a higher reproductive success than local individuals. Overall, we show an origin-dependent reproductive success of dispersing individuals, which illustrates the trade-off between gene flow and local adaptation.



Revisiting genetic load across populations using protein language models

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POSTER SESSION 3 - P03.293, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Genetic load, the cumulative burden of deleterious mutations in a genome, arises from the interplay of mutation, selection, and demographic history. It has been long hypothesized that differences in effective population size (N_e) among human populations influence the efficacy of purifying selection, potentially leading to variation in genetic load across individuals (Motulsky and Vogel 1997). However, studies using non-synonymous mutations as a proxy for genetic load have produced mixed results: some identified higher loads in Europeans compared to Africans, possibly due to post-Out-of-Africa bottlenecks (e.g., (Lohmueller et al. 2008; Tennessen et al. 2012)), while others reported no significant differences (e.g., (Do et al. 2015; Simons et al. 2014)), which may be expected given the demographic history of modern humans (Simons et al. 2014).

Here, we revisit this riddle by taking advantage of recent advances in protein language models, which move beyond simple mutation counts to quantify the functional impact of amino acid changes. By providing a more accurate estimate of genetic load at the individual level, this approach is poised to provide insight into a longstanding question in human genetics and help clarify the roles of demography and selection in shaping genetic variation.

Materials and methods: We obtained phased whole-genome VCFs from 3202 present-day individuals from the high-coverage whole-genome sequencing of expanded Phase 3 of the 1000 Genomes (Bryskia-Bishop et al. 2022). To quantify the deleterious effect of missense variants, we downloaded the dbNSFP database (Liu et al. 2011; Liu et al. 2020), which gathers the functional effects quantified by 34 models. dbNSFP also provides ancestral states inferred from great ape genotypes, as well as genotypes of deeply sequenced Neanderthal and Denisovan samples (Prüfer et al. 2014; Meyer et

al. 2012). We expanded our dataset by including variant effects from pop-EVE (Orenbuch et al. 2023).

To quantify genetic load (d) in each individual, we summed the predicted functional impact scores of all their missense variants, as estimated by one of the models available in dbNSFP. To compare genetic load across human populations, we performed pairwise comparisons of individuals within and between populations and calculated the ratio of their d values. We then tested whether comparisons between populations with different N_e were more likely to yield ratios significantly different from 1. We adjusted for mutation rate differences between extinct and present-day humans by using rates of synonymous substitution.

Results: In the light of previous studies, we hypothesize: (i) present-day populations that experienced bottlenecks may have higher genetic load due to potential differences in selection efficacy; (ii) extinct human lineages, Neanderthal and Denisovan, may have higher genetic load compared to the present-day human populations due to their smaller N_e (Lohmueller 2014); (iii) and finally, utilized models may allow us to assess whether potential differences in genetic load are driven by introgression from extinct human lineages.

Conclusions: Integration of protein language models to variant effect prediction methods transforms our ability to assess the fitness effect of mutations, as they incorporate higher-order information of proteins and do not solely rely on non-synonymous counts. Coupled with the addition of various populations to sequencing databases, genetic load of human populations can be quantified in a more precise approach. This allows us to identify patterns of genetic load across the human populations and further investigate the relationship between observed patterns, demographic histories and selective pressures.



Saving the locals; conservation genomics for the Spanish Toothcarp (*Aphanius iberus*).

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POSTER SESSION 2 - P02.060, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM

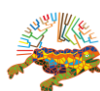
The Spanish Toothcarp (*Aphanius iberus*) is a small fish from the Cyprinodontiformes order, endemic to the Iberian Peninsula and distributed along the Mediterranean coast. It exhibits a great ability to adapt to harsh environments, including high salinity and even desiccation. However, increasing urbanism and agricultural exploitation have fragmented its habitats, leading to a significant population decline. As a result, it was classified as Endangered by the IUCN in 2006.

In this context, despite its wide distribution in Catalonia, it was often thought that these populations were introduced from the south of Spain. Therefore, we decided to address this question using genomic data. We performed whole genome sequencing for 44 *Aphanius iberus* and two outgroups: the Iberian sister species *A. baeticus* and *A. anatoliae* from Turkey. The sampling covered the entire distribution of *A. iberus*, including the populations from southern Spain.

Results indicate that the Catalan populations are well structured, affirming that *A. iberus* is autochthonous to Catalonia. Identifying the distinct genetic populations has been crucial for proposing correct repopulation strategies as well as for identifying introduced populations.

Despite ongoing population declines, when examining genomic variability, we found noticeably high levels of heterozygosity and a low percentage of the genome under Runs of Homozygosity (ROHs). This could be attributed to the species' reproductive and growth strategies.

In conclusion, this study highlights the vital role of conservation genomics in uncovering the genetic diversity and population structure of endangered species like the Spanish Toothcarp. By providing insights into their genetic health and evolutionary history, we can develop more effective conservation strategies to ensure their long-term survival and resilience in the face of environmental challenges.



Selfing VS Outcrossing: Evolutionary Consequences in a Haploid World

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POSTER SESSION 1 - P01.296, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Mating systems - the biological strategies governing how gametes are transmitted across generations - are key drivers of evolutionary dynamics. By shaping levels of genetic variation within and among populations, they profoundly influence how evolutionary forces such as gene flow, genetic drift, and natural selection operate over time.

Bryophytes - mosses, liverworts, and hornworts - offer a unique opportunity to study these evolutionary dynamics. Despite being among the most ancient and diverse groups of land plants, they remain surprisingly overlooked in evolutionary studies. Yet, their biological features make them ideal models to explore how different mating systems affect evolutionary outcomes.

Notably, (i) their life cycle is dominated by a haploid stage (the gametophyte), directly exposing every mutation to natural selection, (ii) they can self-fertilise in two distinct ways, offering novel opportunities to explore the evolutionary consequences of selfing, and (iii) sporophyte emergence (the diploid stage specialized in spore dispersal) is directly linked to sexual reproduction, with direct implications for gene flow and colonisation patterns.

In this project, I will use five pairs of closely related moss and liverworts species that differ in their mating systems (outcrossing vs predominantly selfing) to investigate how different reproductive strategies shape the distribution of genetic diversity within and among populations, influence colonisation dynamics across landscapes, and leave genomic signatures of natural selection.

Because of frequent reproduction through self-fertilisation, we predict greater population differentiation and higher colonisation rates in selfing compared to outcrossing species. Furthermore, due to a reduced effective population size and stronger linkage effects, natural selection is expected to be less efficient in selfers than in outcrossers - both in removing detrimental mutations through purifying selection and in fixing beneficial mutations during adaptation.

This project will provide the first comprehensive look at how selfing and outcrossing shape the evolutionary trajectories of bryophytes. Understanding these processes is essential, not only for appreciating the unique biology of these plants, but also for predicting their responses to rapid environmental changes.



Sexual conflict and sexual pleiotropy

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POSTER SESSION 3 - P03.317, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Males and females often want different things. Natural selection might favour males to be brightly coloured and females to be relatively drab. A male and a female might disagree about whether they should mate with each other. Such tensions are often collectively described as “sexual conflict” and divided into two forms: “intralocus” and “interlocus”. However, this framing quietly accommodates two very different evolutionary processes – genetic constraint and social conflict - under one banner. In the former, male and female fitness are linked through shared genetics, in the latter they are linked through shared social interactions. These two different causal sources – rather than the implied genetic differences – mean that there is no useful definition of conflict that applies across these phenomena. Here, we suggest then that the conceptual and terminological lumping of “sexual conflict” is not justified and even worse, that it has impeded progress by leading to incorrect reasoning, faulty predictions, and wasted research effort. This has become more acute as researchers have explored the mechanistic underpinnings of both

phenomena, with overlapping methodological approaches, and increased focus on the potential interplay between them. Furthermore, a similar lumping of social and genetic coupling of fitness is beginning to spread into other areas, where it is generating similar confusions. We therefore propose that a conceptual uncoupling of “sexual conflict” into true sexual conflict and sexual pleiotropy is required. To achieve this, we: (1) clarify the distinction between conflict and pleiotropy, viewing both through the lens of inclusive fitness theory; (2) show how previous lumping of these phenomena has led to confusions, misdirected research effort, and missed opportunities for scientific advances; (3) detail how that the terminology of “intralocus” versus “interlocus” has added to this confusion, and (4) establish links between these evolutionary tensions and other areas of biology. By disentangling conflict and pleiotropy, we show the potential scientific advances that can emerge across biodiversity, molecular genetics and ecological research.



Sexual conflict over floral behaviour: pollination-induced flower closure is costly to the pollen recipients in a sexually reproducing diploid dandelion

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POSTER SESSION 1 - **P01.371**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Theory of sexual conflict predicts the evolution of manipulative pollen that shortens floral receptivity (e.g., flower closure or wilting), but few empirical studies tested this prediction. The Japanese dandelion, *Taraxacum japonicum*, is a self-incompatible diploid sexual reproducer. We found that pollination induced flower closure in this species, and how quickly flowers closed was affected by the pollen identity; pollen donors show phenotypic variance in their ability to induce flower closure. In addition, naturally pollinated flowers produced more seeds when they received

additional pollen after their flower closure, demonstrating that flower closure is induced despite insufficient pollen load for the pollen recipient. On the other hand, flower heads with fewer seeds produced larger seeds, suggesting the paternal fitness advantage of a low seed set. These results provide evidence for prezygotic sexual conflict in a plant. Furthermore, the fitness consequences of flower closure varied, likely due to the varying abundance of pollinators. Our findings thus shed new light on a less appreciated mechanism by which climate mediates plant reproductive trait evolution.



Socially responsive crickets: insights into the evolutionary consequences of behavioural interactions

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POSTER SESSION 1 - P01.120, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Social interactions shape individual phenotypes and fitness, and can therefore be major drivers of evolution. However, whether variation in social behaviour and plastic responses to social partners (social plasticity) has evolutionary consequences remains unclear. Social plasticity involves both an impact on and responsiveness to partners' phenotypes, and correlations among these components may influence evolution in unexpected ways. Addressing this requires analysing individual-by-social environment interactions within a reaction norm framework. Using this approach, we developed a novel method to quantify individual variation and covariation in social behaviour, responsiveness, and impact in male southern field crickets (*Gryllus bimaculatus*). We focused on agonistic interactions, where individuals adjust aggressiveness based on opponents' body

size—a key factor in fight outcomes. By categorising individuals as “small” or “large” and observing repeated dyadic fights, we assessed size-related variation in average behaviour, social responsiveness, and social impact. Our results reveal significant individual-by-social environment interactions: competitors differed in aggressiveness, responsiveness to opponent size, and impact on opponent behaviour. There was a negative correlation between aggressiveness and social responsiveness, indicating the presence of a possible evolutionary constraint on these traits. This study provides the first empirical evidence of new mechanisms linking social interactions to evolutionary responses to selection, emphasising the need to consider underlying correlations in social reaction norms to predict evolutionary change.



Spawning patterns and symbiotic relationships of Korean bitterling fishes (Pisces: Acheilognathinae) with regard to host mussels

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POSTER SESSION 1 - **P01.231**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Bitterling fishes evolved an extraordinarily unique symbiosis with freshwater mussels, wherein the bitterlings are obligated to spawn in the gill cavities of the host mussels for reproduction. Mussels, in turn, exploit bitterlings as a dispersal vector via attaching their larvae (i.e., glochidia) to the gills, fins, or body of the fish during early life stages. The interaction has thus been considered as ‘mutualistic’, although the opposite view of ‘double host-parasitic’ (i.e., antagonistic) also proposed. Therefore, which interspecific interaction—mutualism, commensalism, or parasitism—best characterizes the bitterling–mussel relationship remains unclear. In this research program, we pursued four specific objectives. First, we determined spawning preferences of seven Korean bitterling species in relation to host mussel’s body size. Second, we tested the hypothesis that bitterlings prefer larger hosts by quantitatively analyzing egg ejection rates of mussels through laboratory-based behavioral experiments. Third, we analyzed the phylogeographic and population genetic structure of two coevolving bitterling–mussel species in a comparative context to evaluate the nature of their symbiotic relationship. Lastly, we investigated potential ecological advantages of autumn-spawning in bitterling fish (*Acheilognathus rhombeus*) and identified candidate genes involved in egg winter diapause through transcriptomic analysis.

Materials and methods: To determine spawning preference of every bitterling fish, we developed a molecular-based species identification technique for discerning bitterling’s eggs and larvae inside the mussels. By conducting behavioral experiments, twenty-seven male-female pairs of *Tanakia signifer* were investigated to assess bitterling’s spawning preference and egg ejection rates of the mussels of different size classes (small vs. medium vs. large). Moreover, we compared the phylogeographic patterns of these two symbiotic species using mitochondrial

DNA variation. Lastly, using RNA-seq we examined the spawning patterns and ecological benefits of autumn spawning behavior, and determined candidate genes contributing to the winter diapause.

Results: Spawned mussels were significantly larger in body size than unspawned mussels, suggesting that bitterlings appear to prefer larger hosts for spawning. Spawning probability clearly increased with mussel size. In behavioral experiments, only 10 of the 27 *T. signifer* pairs successfully spawned: in two small (number of ejected eggs: N = 2), four medium (N = 3), and four large mussels (N = 15). The overall concordance in phylogeographic and population structures between *T. signifer* and *N. breviconcha* may be attributed to their coevolutionary symbiosis. With respect to spring spawner, autumn-spawning behavior of *A. rhombeus* may confer an ecological advantage by allowing the species to preemptively utilize unoccupied mussels. Moreover, we identified the cold shock domain-containing protein C2 (CSDC2) gene as being upregulated during the spawning stage.

Conclusions: Our findings provide some insights into the long-standing evolutionary question of how and why symbiotic interactions between these two taxa have evolved. At least the bitterling-mussel relationship would not be completely mutualistic and the commensalism hypothesis would not be excluded either. The results of this study also shed light on the mechanisms behind the evolution of reproductive traits (e.g., spawning time, egg diapause) and the selective pressures driving counter-adaptations in mussels.

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Strong sexual selection reduces population size, and limits population recovery after heat stress

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POSTER SESSION 3 - P03.325, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Population recovery following environmental stress can depend on demographic structure and evolutionary dynamics. Here, we asked if the eco-evolutionary dynamics of sexual selection can mediate population recovery after exposure to simulated environmental stress in the form of acute increase in temperature. We manipulated the level of sexual selection associated with male aggression in soil mites that are dimorphic for male weapons used in intra-sexual contests. We then examined population dynamics and recovery after heat stress in replicate small and large populations. Our

experiments show that populations facing strong sexual selection have i) reduced population size and stability, ii) greater decline in response to heat stress in large populations, iii) higher rate of growth, and iv) incomplete recovery to the population sizes prior to heat stress. Such reduced population stability and recovery underlines the important role of sexual selection for populations of conservation concern in mediating population dynamics and demographic resilience to environmental change.



Study of coloration diversity with iPACS: A new methodology to link morphology and transcriptomes at the single cell level.

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POSTER SESSION 1 - **P01.248**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Cichlids are a diverse family of freshwater fishes known for their vivid colors and rapid diversification. Despite the visual diversity, only a few types of chromatophores have been described, suggesting that we may be missing much of the true cellular diversity. Chromatophores synthesize pigments (black, yellow, red) or structural elements that reflect light (e.g., guanine platelets that produce iridescent blue), meaning that they have useful optical properties that can be detected without any labeling: just by their natural properties. This makes them an excellent system for directly assaying cellular diversity using high-throughput flow cytometry systems. To study pigment cell diversity, we developed iPACS (intrinsic property analysis and cell sequencing), a multimodal approach that combines flow cytometry, imaging, and single-cell RNA-seq.

Materials and Methods: Chromatophores are rare in skin — less than 3% of total cells — making them expensive to sequence using current technologies. To address this challenge, we used dissociated skin from *Pundamilia nyererei* and sorted cells on the BDS8, a spectral flow cytometer that also captures and computes features from real-time cell images. We gated the chromatophores in two 384-well plates, placing one cell per well. These gates were chosen based on unsupervised analysis of previous BDS8 datasets from several cichlid species. The only dye added was propidium iodide (PI) to exclude dead cells. We also filtered out cell doublets using the image-derived features. Each cell was then sequenced using Smart-Seq, giving us a truly multimodal dataset: cytometry features, an image, and a complete transcriptome for each cell. The BDS8 generates 405 features per

event, including both fluorescence and image-derived parameters. To make the data suitable for downstream analyses, we applied regularization steps and feature selection, with the goal of preserving as much biological signal as possible while reducing redundancy and noise. The images can also be used to train deep learning models for annotation or filtering.

Results: Using iPACS, we propose a methodological approach to evaluate, improve and optimize gating strategies. First, by obtaining transcriptomes for each sorted cell and linking them to cell morphological features, we can retrospectively evaluate how well our gates performed and iteratively improve our gating strategy. Second, the multimodal dataset provides us with an unbiased assessment of cellular diversity at multiple levels of biological organization. For example, using multimodal clustering approaches, we were able to identify six distinct subtypes of melanophores that could not be distinguished by cytometry or transcriptomics alone. This kind of resolution comes from combining all three types of data, and we anticipate that our workflow will contribute to our understanding of the evolution of cellular diversity and how it contributes to the evolution of adaptive coloration.

Conclusions: iPACS allows us to isolate rare chromatophores and begin to uncover and connect their molecular and morphological diversity. It also gives us a powerful framework for future experiments: we can now enrich for specific chromatophore types, improve our gating logic with real data, and begin to compare cellular diversity across species.



Super-shedder vs. super-spreader: from physiology to behaviour

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POSTER SESSION 1 - **P01.365**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Parasite transmission is a key parameter in infection, as it represents the contribution of the parasite to the next generation but also alerts us to potential epidemiological phenomena. Super-spreading is one of them, where a minority of individuals in an infected host population will contribute drastically more to parasite transmission than the average individual. These phenomena are one of the main drivers of disease outbreaks and have been documented throughout the last century across multiple infections. Nevertheless, few empirical studies have been drawn to explain its ecology or physiology in super-spreading and super-shedding. Here, we aimed to discern super-spreading from super-shedding and try to uncover which drivers contribute to each of them.

Hence, we used 16 genotypes of *Caenorhabditis elegans* and *Caenorhabditis*

tropicalis. These include wild-type genotypes from across different geographical locations around the globe and mutants to candidate disease susceptibility/resistance traits. Then, we exposed each host strain to *Leucobacter* celler-infected super-spreader worms and measured a set of fitness (mortality and fecundity), physiological (parasite burden and shedding rate), and behavioral traits (contact rate, symptoms). With this design, we unveiled how much of the transmission of this parasite is driven by shedding (physiology) or spreading (contact) and if both are associated. Besides these findings, we also named a number of traits related to both superspreading and identified host profiles that might lead individuals to super-spread.



Sweeps in the IM model

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POSTER SESSION 3 - With Aperitif, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The impact of positive selection on linked neutral variation is well characterised in panmictic populations. However, selective sweeps may also play a crucial role in local adaptation and speciation, where their effects are less well understood. Strong selection on locally beneficial mutations can promote divergence between populations and nascent species that continue to exchange globally beneficial or neutral variation and is expected to leave strong signatures in population genomic variation. However, genomic inference of local sweeps is often restricted to lossy and limited summaries such as mean pairwise genetic diversity and F_{st} . To address this, we derive

analytical expressions describing the effect of a de novo beneficial mutation on the distribution of genealogies at linked neutral sites under the Isolation with Migration (IM) model. We identify distinct genealogical signatures of positive selection under three evolutionary scenarios: (i) transient local adaptation, (ii) local adaptation leading to a long-term barrier to gene flow, and (iii) adaptive introgression of globally beneficial alleles. These results provide a foundation for developing more powerful methods to detect and characterise selection during speciation.



Testing the link between DNA methylation and phenotypic variation in the water strider, *Microvelia longipes*

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POSTER SESSION 3 - P03.335, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Epigenetics plays a key role in phenotypic response to environmental cues through different mechanisms. To better understand the interaction between phenotypic plasticity and epigenetics, we used whole-bisulfite sequencing in a new insect model system, *Microvelia longipes*, where males show a remarkably exaggerated secondary sexual trait, the third pair of legs. To assess whether DNA methylation is linked to variation in third leg length, we used whole individuals from three highly inbred lines with differences in third leg length. The global methylation level at CpG sites was 12%, one of the highest reported in insects. The majority of methylation occurred in gene bodies, and was not restricted to any specific scaffolds of the genome assembly. CpG methylation clustering was inbred-line dependent, and sex-independent. The highest difference in methylation sites (9,540) was seen between the longest- and shortest-legged lines, suggesting that differential DNA methylation might play a

role in third leg length. To further investigate this in a tissue-specific manner, we subjected individuals of the longest-legged line to nutritional treatment from the second nymphal instar. The individuals fed with rich diet developed significantly longer legs than those fed with poor diet, providing a controlled system to study leg length in the same genetic background. Of the 3,367,541 unique CpG sites from the poor vs rich male third legs comparison, only 24 sites were differentially methylated. Among these, 21 were hypermethylated and 3 were hypomethylated. Twenty-two of these sites were located on chromosome 1914, and 8 of which on *msx2*-interacting protein-like, a hormone-inducible transcriptional repressor. There was no clustering between samples for either nutrition or sex. These results suggest that nutritional input has limited but specific effect on DNA methylation pattern of a highly plastic exaggerated sexually-selected phenotype in *M. longipes*.



The adaptive dynamics of a trait operating over multiple interaction networks

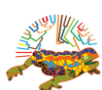
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*POSTER SESSION 1 - **P01.320**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM*

Negative frequency dependent selection is one of the most important mechanisms for maintaining adaptive polymorphisms. When calculating long term trajectories of trait change, we typically operate under the assumptions of small mutational steps and very large (infinite) population sizes. Traditional population genetics approaches rarely allow for frequency dependent effects to be considered, typically only obtaining a selection gradient which is (largely) frequency independent. Adaptive dynamics is a framework through which frequency dependent disruptive selection terms can also be obtained when the selection gradient is 0, enabling the potential for coexistence at this point to be analysed.

However, the adaptive dynamics approach has predominantly been used to analyse well mixed populations where all individuals can be considered to interact over the same network. More recently, there has been an effort to extend the framework to the consideration of structured populations where individuals do not all share the same interaction network: allowing relatedness and other, higher order, network sampling terms to emerge. In such cases it has typically been assumed that a given individual performs all of its interactions over the same network. We studied the effects on the evolutionary dynamics when individuals perform different interactions over different networks.



The consequences of constrained sex allocation under local mate competition

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*POSTER SESSION 2 - **P02.036**, EXPO AREA, August 19, 2025, 5:00 PM - 7:00 PM*

Unmated females in haplodiploid populations may enjoy reproductive success but with the constraint that all their offspring—developing from unfertilised eggs—are male. The presence of such females, constrained to produce only male offspring, is expected to lead to a corresponding female bias being favoured among the offspring of unconstrained females. Previous theory has suggested that the effect is very weak. However, this

conclusion is based upon an inappropriate genetic assumption and a mathematical error. Here, we perform a new theoretical analysis that fully accommodates haplodiploid genetics and corrects the mathematical error, revealing a more substantial impact of constrained females on the sex allocation behaviour of unconstrained females under local mate competition.



The evolution of chemical defence in the mint family - investigating the functional divergence of terpene synthases

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POSTER SESSION 1 - **P01.351**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Plants communicate using volatile blends - concoctions of chemicals that can convey messages of attraction to pollinators or of deterrence to herbivores, and more. Within the Nepetoideae, a subfamily of the Lamiaceae (mint family), species contain extensive chemical vocabularies consisting of various compounds including small molecules called monoterpenoids. In at least two lineages, an insect herbivore-deterrent monoterpenoid, pulegone, is synthesised via the p-menthane biosynthesis pathway, catalysed by several enzymes including a limonene synthase (LS). In *Schizonepeta tenuifolia* (St), the St-LS is located on a biosynthetic gene cluster containing the downstream p-menthane pathway genes, whereas in *Mentha longifolia*, the orthologous pathway genes are mostly dispersed¹². The enzymes involved in the two similar p-menthane pathways appear to have evolved through a mixture of convergent and divergent evolution, but this has not been examined in depth²³. We hypothesise that the LSs evolved convergently, perhaps when the two species adapted to living in the same ecological niche. We also hypothesise that these genes may be under positive selection, and that biosynthetic gene clusters may have influenced their evolution.

Materials and methods: To uncover the functional diversity of LSs across the Nepetoideae clade, we characterised the St-LS orthologs from seven Nepetoideae species by expressing them in vitro in *E. coli*, followed by enzyme assays and Gas Chromatography-Mass Spectroscopy. Then, we phylogenetically mapped St-LS orthologs and produced neutral (3rd codon position) trees to unveil whether these genes are under positive selection. After this, we used synteny analysis to determine which St-LS orthologs are located in p-menthane gene clusters in respective genomes.

Results: Eleven orthologs of St-LS from seven Nepetoideae species were functionally characterised and displayed functionally diverse activity. Most LS orthologs converted geranyl pyrophosphate substrate into multiple monoterpenes including α/β -pinenes, eucalyptol and limonene. Positive selection may be occurring on LS orthologs as differences in topology were observed between phylogenetic trees plotted by full coding gene sequence versus 3rd codon position. Additionally, RNA-seq expression data of an St-LS ortholog from *Hyssopus officinalis* that has β -pinene synthase activity reveals it to be co-regulated with enzymes, that we hypothesise to be part of a novel monoterpenoid pathway.

Conclusions: As the St-LS orthologs have diverse activities, we show that orthology does not guarantee conservation of function. Moreover, the enzymes are multi-product terpene synthases (TPSs), a common feature of other reported plant TPSs in the literature⁴. This may reflect the role of TPSs in volatile blend formation, where multiple compounds are required to convey a tailored chemical message. Meanwhile, TPSs are intrinsically fickle - a single amino acid change can alter the monoterpenoid(s) they produce. These factors, alongside the influence of biosynthetic gene clustering, constructs a complex story for LS evolution in the Nepetoideae clade. Using synteny analysis, we will reveal the genomic location of Nepetoideae LSs. Unravelling the movement of LSs over evolutionary time will aid our understanding of how genes congregate into biosynthetic gene clusters.

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The evolutionary impact of migration on genetic and cultural differentiation

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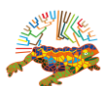
POSTER SESSION 1 - **P01.270**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Humans are shaped by two inheritance systems: genetic inheritance, which follows well-established evolutionary principles, and cultural inheritance, which transmits traits such as language, behaviours, and beliefs. Cultural evolution shares similarities with - but also differs in key ways from - biological evolution, in particular in the way cultural traits are transmitted. An important mechanism shaping cultural dynamics is frequency dependent transmission, where individuals are more or less likely to adopt traits based on their prevalence in the population. In particular, conformity (the disproportionate tendency to acquire the most frequent trait) promotes cultural homogenisation and anti conformity (the disproportionate tendency to acquire the least common trait) can maintain or even increase cultural diversity.

Demographic processes are of particular interest because they can affect both inheritance systems. Migration, for example, has shaped both cultural, and genetic diversities throughout human history. In population genetics it is well established that even very small rates of migration between populations can lead to homogenisation of their genetic composition. However, similar results regarding cultural composition are largely absent. To bridge this gap, we develop a simulation model that describes the joint evolution of genetic and cultural diversity under different scenarios of frequency dependent transmission and migration between two populations. This setup allows us to examine how the same demographic process can have distinct effects on two parallel evolutionary processes—genetic and cultural—occurring within the same species. This approach allows us

to determine when, if ever, we should expect genetic and cultural differences between groups to be similar. In other words, we derive expectations for when migration has a similar, or different, effect on between-group genetic and cultural diversity. We identify cultural transmission and migration scenarios that lead to a faster or slower cultural homogenisation process, or the maintenance of diversity between groups, compared to the genetic process. This, in turn, provides a theoretical comparison of the difference in speed of cultural and genetic change induced by migration.

Our findings contribute to a better understanding of how migration can influence cultural evolution. They provide insight into why some populations maintain strong cultural distinctions despite high levels of gene flow, while others exhibit parallel patterns of genetic and cultural homogenisation. Our results suggest that migration alone is not sufficient to predict cultural similarity between populations, the mode and strength of cultural transmission must also be considered. We show that the "one migrant per generation" rule from population genetics does not apply to cultural traits. Migration and conformity can interact to either promote or prevent homogenisation: in some cases, even high levels of migration fail to erase cultural differences, while in others, conformity amplifies the homogenising effect of migration. Furthermore, our results inform ongoing debates about the relative pace of cultural versus genetic evolution, showing that cultural change is not inherently faster, but depends on the interplay between transmission mechanisms and demographic processes.



The evolutionary predictability of avian colouration under natural and sexual selection

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POSTER SESSION 1 - **P01.367**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The animal kingdom displays a striking diversity of colours and understanding how both natural and sexual selection have shaped this colour gamut is a longstanding focus of evolutionary research. However, we currently lack a predictive understanding of how animal colouration evolves under multifarious selection pressures. Here, we address this in birds by directly quantifying the predictability of plumage colour evolution and investigating how factors capturing variation in the strength and direction of natural and sexual selection among species are related to the evolution of specific colours. Our machine learning approach combines UV-Vis plumage colour data collected from images of museum

specimens for 6643 species with detailed information on avian sexual, social, and ecological traits to quantify the predictability of avian plumage colouration. On average, our models are able to predict species' plumage colouration with >63% accuracy, with ~12% of this predictability uniquely attributable to relationships between colour and its predictors. We also reveal substantial heterogeneity in both the predictability of colour and the relative importance of different drivers of colour evolution across different body regions and between the sexes. Overall, our results provide important new insight into the relative predictability of colour evolution under both natural and sexual selection.



The genetic dynamics of Red Queen coevolution extracted from sediment cores

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POSTER SESSION 1 - **P01.360**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Co-evolution is instrumental to the maintenance of genetic diversity, shaping the evolution of populations and species. Antagonistic co-evolution is prolific in host-parasite interactions, where benefit gained by one antagonist comes at the cost of the other. Historical sediments provide a powerful method for studying past host-parasite populations and track their co-evolution over time. This study investigates the genetic signal of host-parasite interactions by using sediment cores to establish a timeline of host-parasite genotype history in a focal population.

The water-flea *Daphnia magna* and its bacterial parasite *Pasteuria ramosa* are a model host-parasite system that has been studied extensively on the topic of antagonistic co-evolution. In this system, infection is specific to host-parasite genotype combination; previous studies have shown that Red Queen dynamics are prevalent in this system. We track infectivity genes in the parasite *P. ramosa* and resistance genes in its host *D. magna* with

microsatellite markers over several decades, using host and parasite resting stages that have been chronologically archived in the sediment of a focal pond. These resting stages have been retrieved by extraction of sediment cores from the pond and analyzed with Fragment Length Analysis.

We find that infectivity- and resistance genotype frequencies fluctuate over time with a high amplitude in both antagonists. These oscillating genotype dynamics indicate negative frequency dependent selection within the host- and parasite populations, and are characteristic of antagonistic co-evolution. By investigating the long-term dynamics of co-evolution between *D. magna* and *P. ramosa* on a genomic level, we shed light on the evolutionary interactions shaping this model host-parasite association, and demonstrate how historical genetic material can be used to investigate co-evolution and the maintenance of genetic diversity.



The Hormonal Control Of Regeneration And Reproduction In The Marine Annelid *Syllis Prolifera*

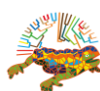
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POSTER SESSION 1 - **P01.362**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Annelids or segmented worms have been traditionally used as models to study two important processes in developmental biology: sexual reproduction and regeneration. These two events are intimately linked in the very well-known annelid *Platynereis dumerilii*, and their control seems to occur through a brain hormone. This species is able to grow and regenerate during most of its life due to the secretion of methylfarnesoate, a hormone that stops when the worms undergo sexual maturation, suffering dramatic modifications to become reproductive individuals that will die shortly after spawning. However, other species seem to have co-opted the process by metamorphosing only their posterior end to commit it for sexual reproduction, in a process called stolonization. This is the case of the marine annelid *Syllis prolifera*, traditionally reported to present two different and negatively related hormones controlling both processes: while a gut hormone would

allow the normal worm growing and regeneration, a brain hormone would stop that secretion letting sexual reproduction to start. Once it is finished and the stolons are detached, the secretion of the gut hormone would be back and the posterior part would be rapidly regenerated. However, a series of laboratory experiments in *S. prolifera* have showed us unexpected results that questioned traditional hypotheses about the hormonal control of regeneration and reproduction in this species. First of all, individuals are still able to regenerate while reproductive individuals are produced in the posterior end. In addition, if the animals are cut just after the stolons detachment and the regeneration of the posterior end, they are not capable to secondly regenerate, and they eventually die. These results allow us to hypothesized on the existence of two different pathways of annelid regeneration, which can influence each other, but appear to operate in a separate way when the animal is sexually developing.



The interplay of sexual selection and hybridization can drive sexual radiation

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POSTER SESSION 1 - P01.117, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Many evolutionary radiations show spectacular interspecific variation in sexually selected male displays, and the formation process of such sexual radiations has been a major puzzle in evolutionary biology. Based on evolutionary simulations, here we propose that an interplay of sexual selection and hybridization can drive the rapid formation of multiple species with diverse exaggerated sexual displays within a lineage. Existing theories suggest that sexual selection can cause evolutionary dynamics with multiple stable equilibria, with which multiple species with distinct sexual displays and mate preferences can persist stably. However, the stability of each evolutionary equilibrium hinders the formation of new species from an existing

species that is already occupying one of the stable equilibria. In such situations, hybridization between phenotypically similar but genetically distinct lineages can catalyze speciation. Our simulations demonstrate that genetic variation arising from hybridization can trigger the evolution of sexually selected displays toward previously unoccupied stable equilibria, forming a new species with a novel sexual display. A key condition for this speciation mechanism is that the increased genetic variation in hybrid populations creates inter-individual variation in mate preference to modify the sexual selection regime. We discuss future directions for assessing whether this condition can be satisfied in real organisms.



The joint influence of incompatibilities and heterosis on hybrid population genetics

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POSTER SESSION 3 - **P03.263**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

A long-standing question in evolution is whether speciation can occur despite hybridization or because of hybridization. Intuitively, heterosis—the increased fitness of hybrids compared to their parents—can facilitate introgression and weaken barriers to gene flow. On the other hand, hybrid incompatibilities can hinder introgression and strengthen barriers to gene flow. However, in the context of a hybrid population formed in a new environment, heterosis could jump-start adaptation through increased diversity, whereas hybrid incompatibilities could hinder population expansion. Few models have explored the joint effects of heterosis and incompatibilities in hybrid populations occurring in a new environment under these contrasting expectations.

Here, we investigate the long-term genetic composition of a hybrid population where both hybrid incompatibilities and heterosis occur. Specifically, we use a continent-island model with a Dobzhansky-Muller incompatibility (DMI) between two loci that also experience overdominant heterosis. We determined the equilibrium allele

frequencies that describe the long-term genetic composition of the hybrid population and how these are affected by the model parameters.

Combining analytical and numerical methods, we find that the recombination rate and the relative strength of DMIs and heterosis are key parameters determining evolutionary outcomes. Strong DMIs break the symmetry in the equilibrium frequencies of incompatible alleles driven by heterosis, leading to one parental genome dominating the hybrid population. When recombination is rare, stronger DMIs are needed to break the symmetry. Moreover, neutral diversity neighboring the selected loci varies non-monotonically along the genome depending on the strength of DMIs and initial admixture fractions.

Our results reveal how the complex interaction of heterosis and hybrid incompatibilities leads to a wide range of evolutionary outcomes regarding the maintenance of polymorphisms, the strength of barriers to gene flow, and ultimately, the probability of speciation.



The link between pace and shape of ageing: a comparative analysis in birds and mammals

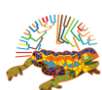
Ronget V¹

¹Johannes Gutenberg University

POSTER SESSION 3 - P03.299, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

In his influential paper on the evolution of senescence, Hamilton demonstrated that the force of selection should always decrease with age and predicted, as a consequence, that senescence should be pervasive across species. While comparative analyses of senescence patterns have demonstrated overwhelming evidences of senescence affecting survival and fertility independently in mammals and birds, no study has focused on using Hamilton's force of selection for comparative purposes. In this presentation, we fill that knowledge gap by computing the force of selection on survival for 72 wild species of birds and mammals and developing a new framework to compare the forms of decrease in the force of selection using a pace of life standardization. We

highlight that fast and slow species display markedly different shapes, with fast species associated with an earlier decrease in the force of selection during their lifetime, while in slow species, the decrease starts slower but becomes steeper later in life. As expected, a very low percentage of individuals were still alive at the stages where the force of selection was low for most species; however, some slow species showed that a non-negligible proportion was still alive at this stage, including both menopausal and non-menopausal species. Our results demonstrate a strong relationship between the pace and shape of senescence across species and shed new light on the evolution of aging, resulting in the diversity of demographic trajectories observed in wild species.



The performance of phenotypic evolutionary rate inference methods using continuous multivariate data: a simulation approach

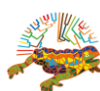
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POSTER SESSION 3 - **P03.313**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Uncovering rates of phenotypic evolution provides insights into the evolutionary patterns and mechanisms giving rise to phenotypic innovation and novelty. Continuous phenotypes are a compelling data source for these types of analyses because they provide a highly quantitative and objective picture of trait evolution, however they also introduce computational challenges, and existing methods have been shown to provide conflicting results. Here, we investigate how various methods perform at inferring the continuous phenotypic rates of evolution on in silico datasets simulated with a mechanistic diversification (birth-death) model and with continuous traits set to evolve independently under Brownian motion at different rates (variances). This produced a full time calibrated tree, for which true absolute rates and rate shift points for each trait and all traits combined were known. The trait values generated at the tips were then used as input continuous variables to test the performance of existing rate inference approaches in their ability to recover the original (true) trait rates. We focused on methods that enable multivariate data and branch-by-branch rate variation: two phylogenetic comparative methods (PCMs) – RRphylo, a phylogenetic regression approach, and Bayes Traits a Bayesian approach based on a multivariate Brownian

motion model (mvBM) of evolution—and include RevBayes, a flexible Bayesian phylodynamic method that can infer multiple phylodynamic parameters at once. Finally, we tested each program using different tree sizes, with phylogeny subsampled from the full tree (at 50% and 25% sampling) to test their robustness to tree size and incomplete taxonomic sampling. Our results demonstrate that while RRphylo more readily detects stochastic fluctuations in the rate of evolution along individual branches, Bayes Traits and RevBayes tend to infer the underlying rate regimes well but failed to recover smaller rate fluctuations. Our results also suggest that there is a trade-off between subsampling and tree size, especially for Bayes Traits which, in our analyses, failed to reliably converge for tree sizes of > 300 extant species. Finally, we show multiple examples of each method correctly identifying the position of rate shifts while inferring the wrong direction of those shifts, suggesting that inferring the direction of rate shifts of continuous traits may be a more challenging problem. This study highlights important considerations for phenotypic evolutionary rate analyses including the desired granularity of inferred rates, dataset size and convergence, and accurately capturing both the location and direction of rate shifts.



The Population Genetic History of the Hittite Capital Hattusa

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POSTER SESSION 3 - P03.273, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Boğazköy (Hattuša), the capital of the Hittite Empire (ca. 1650–1200 BCE), has been a settlement occupied for multiple millennia, with archaeological layers extending from the Chalcolithic to the Ottoman period. Located in northeastern central Anatolia, Boğazköy represents a key intersection of local and interregional dynamics in prehistoric and historic Anatolia.

Among the various populations that inhabited the site, the Hittites stand out not only for their political and cultural characteristics but also for their linguistic significance as the earliest attested speakers of the Indo-European languages. Thus, studying ancient DNA from Boğazköy may offer a unique opportunity to explore proposed hypotheses regarding the spread of Indo-European languages across Eurasia. We analyzed genome-wide data from 21 individuals, 4 previously published and 17 newly sequenced genomes, spanning the Early Bronze Age to the Ottoman period. Using state-of-the-art population genetics methods, we examined patterns of genetic continuity, admixture, and population structure within Boğazköy and in its surrounding regions.

Preliminary results indicate genetic homogeneity across the Boğazköy individuals over time, suggesting long-term population stability despite historical and

political changes. However, one individual from the Roman period stands out as a potential genetic outlier, suggesting long-distance mobility from possibly the Caucasus.

Interestingly, the genomes associated with the Hittite period do not show robust evidence of Pontic Steppe-related ancestry, despite the spread of Indo-European languages often being associated with Pontic Steppe-related migrations. This observation raises multiple possibilities: early Indo-European expansions into Anatolia may have involved cultural transmission rather than large-scale population movement, or genetic admixture may have been limited to elite classes without widespread impact on the broader population. Alternatively, Pontic Steppe ancestry may have been diluted over time through interactions with local Anatolian populations.

Our study contributes to understanding the demographic history of central Anatolia by contextualizing local population dynamics within broader evolutionary processes such as migration, admixture, and population structure change. These findings highlight how Boğazköy served both as a stable population hub and as a centre for interregional interaction, providing insights into how continuity and mobility shape regional gene pools over millennia.



The role of ongoing introgression on diversification in an adaptive radiation of sailfin silverside fishes from Sulawesi

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POSTER SESSION 3 - **P03.340**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Adaptive radiations are excellent study systems for understanding the mechanisms of rapid species diversification. While these radiations contribute significantly to the world's biodiversity, much remains unknown about the genetic and ecological factors underlying these rapid successions of speciation. One potential driver of such adaptive diversification is introgression at the root of the radiation, which can introduce genetic variation that fuels ecological and phenotypic divergence. However, not only introgression at the base, but also ongoing gene flow into and between members of a radiating flock may play an important role in creating, maintaining, or collapsing species diversity.

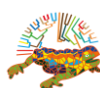
The sailfin silversides (Telmatherinidae) of the Malili Lakes in Sulawesi, Indonesia, represent a classic example of adaptive radiation, comprising around 20 species, each adapted to specific ecological niches. Our genomic analyses, based on whole genome sequences, revealed introgression at the base of this radiation, as well as ongoing gene flow both within the lacustrine species flock and with a closely related riverine lineage, *Telmatherina bonti*.

Ongoing introgression among lake species indicates incomplete reproductive isolation

and continued speciation. Additionally, multiple lacustrine species receive gene flow from the same riverine source, raising the question: does this shared introgression homogenise the genomes of the lacustrine species, or does it contribute to their divergence?

We examine the fate of introgressed alleles across the genomes of lacustrine species by identifying genomic regions originating from riverine introgression and examining signals of selection on these introgressed regions. Our aim is to determine whether shared alleles are neutral, purged, or under divergent selection across species. Preliminary findings suggest that some introgressed alleles may follow species-specific evolutionary trajectories, potentially reinforcing rather than eroding species boundaries.

This work highlights how ongoing gene flow from a common external source can shape diversification dynamics in adaptive radiations. It challenges the assumption that introgression necessarily constrains speciation and instead suggests that, under certain conditions, it may facilitate creative evolutionary processes.



The SLC30A9 gene case: Testing Denisovan Adaptive Introgression using Approximate Bayesian Computation

Garcia-Calleja J¹, Lao O¹, Bosch E¹

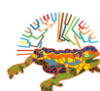
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POSTER SESSION 3 - P03.280, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Strong signatures of positive selection have been consistently identified around the human SLC30A9 gene, particularly in East Asia, with a methionine-to-valine substitution in the encoded zinc transporter proposed as a putative adaptive variant. Our previous work demonstrated a differential molecular phenotype in zinc handling by the mitochondria and endoplasmic reticulum, impacting mitochondrial metabolism. Notably, all high-coverage Neanderthal genomes carry the methionine variant, whereas the Denisovan genome is homozygous for the valine variant. Furthermore, we showed that the putatively selected modern human SLC30A9 haplotype exhibits striking similarity to the Denisovan sequence over 70,614 bp. While we ruled out incomplete lineage sorting as producing this unusual configuration, the presence of this haplotype in the YRI population at up to 7% frequency complicated any direct inference of adaptive introgression from Denisovans.

Here, we applied the Approximate Bayesian Computation (ABC) statistical framework to explore the most plausible evolutionary

origin of this methionine-to-valine substitution and to estimate both the timing of the mutation and the selection coefficient in different human populations. Using forward simulations, we modelled three potential scenarios for the origin of the substitution: Denisovan, Neanderthal, or ancestral human, considering Back-to-Africa migrations in each case. Moreover, we used a variety of summary statistics that account for linkage disequilibrium, haplotype and nucleotide diversity, and admixture to compare the patterns of variation present in each of the simulated datasets to that of the observed data. Our results indicate a higher posterior probability for a Denisovan origin and strong selection coefficients for East Asian and European populations. Thus, following an adaptive introgression event in East Asia, the substitution likely spread to European populations, being later introduced to Africa. Given the critical role of the mitochondria in skeletal muscle thermogenesis, we speculate that adaptation to cold may have driven this putative adaptive introgression event.



The tempo and mode of toxicant sensitivity evolution

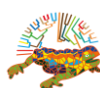
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POSTER SESSION 3 - P03.311, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Chemical pollution is a critical driver of biodiversity loss, impacting ecosystems worldwide. Species differ remarkably in their sensitivities to environmental toxicants, and understanding why is crucial to better predict ecological risks and inform conservation strategies. By considering effect concentrations (the concentration of toxicant X that produces effect Y in species Z) as quantitative traits in a macro-evolutionary perspective, we investigate how toxicant sensitivity evolves across diverse taxa. Here, we use large ecotoxicological databases of standardised effect concentrations (>1

million empirical results) for a wide variety of toxicants and species. Combined with time-calibrated molecular phylogenies, we use a data-driven phylogenetic comparative framework to elucidate the processes and patterns that have given rise to contemporary distributions of species' sensitivities. Our analysis revealed that the tempo and mode of toxicant sensitivity evolution differed across classes of toxicants, and recovered cases of convergent evolution across deeply diverged taxa.



Timing is everything: Evolution of ponerine ants highlights how dispersal history shapes modern biodiversity

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POSTER SESSION 1 - P01.369, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: A cornerstone of biogeography since its early developments has been to disentangle the drivers of the distribution of biodiversity patterns worldwide. Three main hypotheses compete to provide explanations for differences in species richness across bioregions: (1) differences in diversification rates (Diversification Rate Hypothesis), (2) differences in timing of colonization (Time-for-Accumulation), and (3) differences in dispersal flux (Asymmetric Dispersal Hypothesis). Ponerine ants, a diverse cosmopolitan subfamily of predatory ants with a major role in trophic chains of ecosystems, presents an ideal model group to provide an invertebrate perspective on macroevolutionary drivers of global biodiversity. Although previous work suggested a Neotropical origin for the group, conflicting fossil evidence and limited sampling have left their biogeographic and evolutionary history unresolved.

Materials and methods: Here we produce the first complete species-level phylogeny of the subfamily of ponerine ants (Formicidae: Ponerinae) based on new UCE sequencing and taxonomic grafting covering the 1,534 currently identified taxa. We combine phylogenetic results with a large-scale geographic database to reconstruct the diversification dynamics and dispersal history of extant ponerine ants. We designed a new test for the Diversification Rate Hypothesis that operates at any time step along evolutionary time. We mapped the timing of colonization of geographic areas providing a qualitative assessment for the Time-for-Accumulation hypothesis. Finally, we estimated the origin of lineages in bioregions contrasting between in situ speciation and dispersal events to weigh the importance of

those processes in shaping biodiversity over time.

Results: We showed that extant ponerine ants originated in Gondwana, spread primarily eastward across tropical bioregions, and then more recently expanded toward temperate areas. Differences in the timing of colonization events were identified as the prominent key drivers of present-day biodiversity patterns with most of the diversity localized in the Tropical areas. In addition, differences in diversification rates and asymmetrical dispersal histories acted both as buffers of the heterogeneity in biodiversity. Indeed, we unraveled a longitudinal asymmetry of dispersal events over time mostly from West to East. This imbalance in dispersal flux played a part in mitigating differences in diversity expected from the Gondwanan ancestry of the group. Similarly, within the tropical belt, we identified higher diversification rates in the eastern Indomalaya and Australasia that fueled the accumulation of lineages in these more recently colonized bioregions.

Conclusions: These results suggest that tropical niche conservatism played a major role in shaping the biogeographic and evolutionary history of Ponerinae with most lineage diversification and dispersal occurring within the Tropical belt. The dominance of the Diversification Rate Hypothesis in explaining the current distribution patterns highlights the general importance of considering the timing of past biogeographic and evolutionary events, in complement to the scrutiny of differences in current diversification rates, to gain a deeper understanding of Earth's biodiversity patterns.



Transition from monogamy to polygamy in human-modified environments: the case of free-ranging dogs

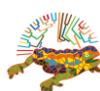
Car C¹, Adavoudi R¹, Berghänel A², Vanderheyden M², Moura A¹, Range F², Cimorelli G², Lazzaroni M², Dale R³, El Berbri I⁴, Spatola G^{5,6}, Mousseau T⁶, Marshall-Pescini S², Pilot M¹

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POSTER SESSION 1 - P01.370, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Unlike all wild canids, which are characterized by socially monogamous mating systems, domestic dogs (*Canis lupus familiaris*) are polygamous. This transition from monogamy to polygamy is often considered a consequence of selective breeding but polygamy may have instead appeared at an early stage of dog's commensal relationship with humans. However, the specific characteristics of this mating system have rarely been examined, which prevents a critical evaluation of this hypothesis. Critically, knowledge of the genetic mating system and its determinants is still limited, despite several observational studies describing copulation patterns in dog populations. We used genome-wide SNP genotypes (>120,000 SNPs), to characterise in detail, the genealogies of 526 free-ranging domestic dogs from three populations in Italy, Ukraine, and Morocco. Additionally, social network based on extensive behavioural data for the Moroccan population, was compared with reproductive success data extracted from the genealogies. All three populations presented polygynandrous mating systems as expected, however we also found that mate choice was not random, with clear differences in the distribution of reproductive partners compared with simulated genealogies modelling random

mate choice. Comparison with behavioural data showed a preference for familiar mating partners, as reproductive success was correlated with the strength of social interactions and the number of connections with individuals of the opposite sex. Contrary to wolves, we observed frequent retention of kin individuals within social groups and reduced incest avoidance, because the three populations showed similar or higher number of incest compared to random expectations. Several lines of evidence indicated sexual selection for larger males, resulting in sexual size dimorphism. Nevertheless, multiple paternity within litters was identified, suggesting that females often mate with multiple males within one oestrus, which implies the potential for post-copulatory selection, potentially involving sperm-competition mechanisms. The shift in mating system during domestication, may have reduced gene flow from wolves during early domestication stages, and thus facilitated the emergence of domestication traits in dogs. The first populations subject to selection by humans were likely polygamous proto-dogs rather than monogamous wolves, and therefore future studies on dog domestication should consider the mating system as a potential driver of dog evolution.



Transposable element copy number variation reveals their evolutionary dynamics in apple domestication

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POSTER SESSION 3 - **P03.341**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Transposable elements (TEs) are repetitive DNA sequences capable of moving within the genome, contributing to genome plasticity, gene regulation, and phenotypic diversity. They are involved in controlling key agronomic traits and may promote rapid adaptation to environmental changes. TEs can influence gene expression by introducing or modifying transcription factor binding sites (TFBS), thereby impacting regulatory networks. Although abundant in plant genomes, the population-level dynamics of TEs remain poorly understood, particularly in long-lived perennials such as fruit trees. The cultivated apple (*Malus domestica*) is a valuable model for studying TE evolution due to its well-documented domestication history, involving *M. sieversii* as the primary progenitor and introgression from *M. orientalis* and *M. sylvestris*. Despite TEs comprising about 50% of the apple genome, their role in domestication remains unknown. A key unresolved question is whether TE copy number variation (CNV) across *Malus* species reflects neutral demographic processes, adaptive divergence, or both. Answering this would offer important insights into the evolutionary significance of TEs and their impact on domestication and adaptation.

Materials and methods: We generated a comprehensive TE consensus library for the *Malus* genus using de novo predictions from 35 short-read sequenced genomes representing wild and cultivated accessions. A dedicated pipeline was developed to estimate TE CNV from resequencing data of 190 *Malus* individuals spanning both wild and domesticated lineages. Using this dataset, we implemented a statistical framework to distinguish TEs associated with neutral population structure ("population structure TEs") from those showing independent variation ("residual TEs"). We compared the distribution of these insertions across five *Malus* reference genomes using the panTE library. Gene ontology and TFBS enrichment analyses were performed to assess the regulatory potential of residual TEs. We further examined 73 *M. sylvestris* individuals sampled across Europe to

test for associations between residual TE abundance, geographic coordinates, and bioclimatic variables using generalized linear models.

Results: A total of 158,331 unique TE insertions were identified. Of these, 2,341 correlated with SNP-based neutral genetic structure and were classified as population structure TEs, while 1,299 showed no correlation and were defined as residual TEs. Comparative analysis revealed that structure TEs were generally older than residual TEs. Among the reference genomes, 630 structure TEs were shared across all five species, whereas only 121 residual TEs were common, suggesting greater species specificity among residual elements. In *M. sylvestris*, 36 residual TEs were significantly associated ($p < 0.05$) with both geographic and climatic variables, indicating a potential role in local adaptation. These included three TIR-type DNA transposons, eight LTR retrotransposons without superfamily classification, six Copia, and 19 Gypsy elements. These TEs were significantly enriched in TFBS motifs. Gene ontology analysis revealed that 1,164 insertions derived from these 36 TE families were located near genes involved in stress responses.

Conclusions: Our study demonstrates that TE CNV captures both demographic structure and adaptive signals in apple populations. Using short-read resequencing and a dedicated pipeline, we identified 36 residual TEs significantly associated with geographic and climatic variation in *M. sylvestris*. These elements are enriched near stress-related genes and regulatory motifs, suggesting a role in adaptation. This represents the first population-specific TE analysis in apple, offering a comprehensive perspective on TE dynamics. The resulting dataset also provides an unprecedented resource for future research on TE evolution and their contribution to adaptation in fruit trees and other perennials.



TreeProfiler: Computing Phylogenetic Profiles and Visualizing Metadata on Large Trees

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POSTER SESSION 3 - **P03.315**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Phylogenetic profiling is a computational approach that explores the presence or absence of traits across gene or species phylogenies. Originally proposed to infer functional associations between proteins based on co-evolutionary patterns, it has evolved into a versatile tool for mapping evolutionary distributions of traits across genomes. Applications now span gene function inference, detection of horizontal gene transfer, identification of synapomorphic traits, and comparative analyses in microbial ecology.

Despite its broad utility, phylogenetic profiling faces challenges when applied to the large and complex trees generated by modern genomics. Most tools are limited to annotating leaf nodes, hindering trait propagation to internal nodes. Visualization tools struggle to handle multiple traits simultaneously or to scale to trees containing tens of thousands of nodes. Many tools also rely on static images or require manual scripting to configure layouts and annotation types, creating a bottleneck for exploratory analyses. Integrating diverse metadata further increases complexity and limits accessibility for users lacking programming skills. Internal node annotation is essential for summarizing traits at ancestral levels, enabling analyses of lineage-specific patterns, ancestral character reconstruction, and phylogenetic signal estimation.

To address these limitations, we developed TreeProfiler, a software tool for computing phylogenetic profiles and visualizing metadata across large phylogenies. TreeProfiler automates annotation from diverse data sources including functional annotations (e.g., EggNOG, KEGG), protein domain architectures (Pfam, SMART), taxonomic identifiers (NCBI, GTDB, mOTUs), and multiple sequence alignments. It extends annotation beyond leaves to internal nodes through data-type-aware summarization for numeric, categorical, and boolean data types. TreeProfiler further supports analytical methods including ancestral character reconstruction, phylogenetic signal testing, and lineage specificity calculations to enrich internal node annotations with evolutionary metrics.

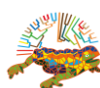
TreeProfiler provides both a command-line interface and a graphical user interface, each enabling users to annotate diverse metadata onto phylogenetic trees, propagate and summarize annotations at internal nodes, and visualize annotated trees with

predefined and customizable layouts. The command-line interface includes two subcommands, `treeprofiler-annotate` and `treeprofiler-plot`, for metadata annotation and visualization respectively. The graphical interface integrates these functionalities into an interactive environment, allowing annotation, layout configuration, metadata filtering, and dynamic exploration of large trees without scripting.

TreeProfiler combines scalable visualization of very large trees with internal node summarization and a flexible layout system that supports dynamic rendering of multiple metadata types. It is built on the ETE Toolkit v4 visualization engine, leveraging its efficient rendering architecture to enable interactive exploration of large phylogenies with tens of thousands of annotated nodes. TreeProfiler allows numerical traits to be visualized as heatmaps, bar plots, or color gradients; categorical traits as colored labels, pie charts, or stacked bars; and presence-absence matrices for binary annotations.

We demonstrate scalability by annotating the mOTUs reference taxonomy tree, comprising 124,295 archaeal and bacterial genomes. Visualization integrates a heatmap summarizing habitat-specific abundance profiles across 20 environments, automatically summarizing data at internal nodes and dynamically adjusting the matrix layout to zoom level (https://github.com/dengzq1234/treeprofiler_paper/blob/main/fig3_motus_full_habitat/fig3_motus_full_habitat.png). TreeProfiler retains interactive visualization at this scale, supporting exploration from phylum-level patterns down to individual genome-level details.

TreeProfiler bridges current gaps in phylogenetic profiling by combining automated metadata annotation, internal node summarization, and scalable visualization in a single platform. It facilitates exploration of evolutionary and ecological patterns in large phylogenies while reducing technical barriers. It is freely available at <https://github.com/compgenomicslab/TreeProfiler>. Code and instructions for reproducing all figures are provided at https://github.com/dengzq1234/treeprofiler_paper/. A preprint is available on bioRxiv (<https://doi.org/10.1101/2023.09.21.558621>).



Triploidy in parasitic worms: an overlooked aspect in host-parasite dynamics and evolution of drug resistance?

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POSTER SESSION 3 - **P03.248**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Parasitic worms significantly affect human health, infect important livestock breeds, and cause serious crop losses. Thus, they attract the attention of researchers from various fields. Parasitic worms are assumed to reproduce sexually, which should be advantageous to counter the evolution of resistance in the host populations. Sexual reproduction should also allow faster evolution of drug resistance in the parasite population. However, some studies report triploid populations of parasitic worms. For example, *Paragonimus westermani*, a trematode infecting livestock and humans. Triploidy is usually associated with asexual reproduction, raising interesting questions about the role of triploidy in parasite evolution, specifically, host-parasite coevolution or drug resistance. To explore such questions, we require background knowledge on the distribution of triploidy, reproductive modes of triploids, and potential costs or benefits of triploidy. To the best of our knowledge, no prior review has focused on these aspects. Here, we aim to fill such a gap by conducting a systematic review on triploidy of parasitic worms.

Materials and methods: We conducted a systematic literature review using Web of Science and Scopus. Our search combined keywords related to triploidy ("triploid*", "autotriploid*", "allotriploid*") with taxonomic terms covering parasitic worms from Platyhelminthes, Nematoda, and Monogenea, including scientific and common-name synonyms. To get a better overview of the origin of triploidy, we also included taxonomic keywords covering free-living flatworms.

The combined search (after checking for duplicates) returned 416 papers. After initial abstract and full-text screening, 100 studies were selected as relevant. From these papers, whenever possible, we extracted species names, reproductive system and mode, coexistence with other ploidy levels, and origin of triploidy.

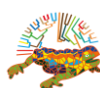
Results: We identified 54 triploid species: 37 flatworms (15 parasitic), 16 roundworms (14

parasitic), and one Monogenea species. Parasitic flatworms include species such as *Fasciola hepatica*, *P. westermani*, and *Schistosoma mansoni*, species important for human and livestock health. Nine triploid roundworms belong to the *Meloidogyne* genus, a group of root-knot nematodes causing significant crop losses. Two triploid species (*Haemonchus contortus* and *S. mansoni*) are known to have populations resistant to drugs, although it is unclear whether there is a connection between triploidy and drug resistance. Several studies report triploid individuals of *P. westermani* developing faster, producing larger eggs and larvae, and having larger body size compared to diploids.

Most triploid individuals were reported to be parthenogenic, with a few exceptions among free-living flatworms that can switch to fully functional sexual reproduction. At the same time, few studies concentrated specifically on the mating systems of triploids.

Conclusions: Here we report the first systematic review of triploidy in parasitic worms across three phyla, Platyhelminthes, Nematoda, and Monogenea. Only 30 parasitic species are found to have populations with triploid individuals. This may reflect the rarity of triploidy, as well as a lack of interest in triploidy, partially due to the general assumption that asexual reproduction connected with triploidy is an evolutionary "dead end."

However, triploidy may alter life-history traits relevant to the evolution of host-parasite interaction and human health. Another direction worth investigating is a connection between triploidy and the evolution of drug resistance, especially in terms of how triploidy affects mating systems and genetic architecture. With this review, we wanted to draw attention to the potential role of triploidy in parasite evolution and encourage further investigation in this direction, both theoretical and experimental.



Universality and Cultural Specificity in Artifact Evolution: A Cross-Cultural Transmission Chain Experiment

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POSTER SESSION 3 - P03.290, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: The capacity for cumulative cultural evolution is a hallmark of the human species, enabling adaptation to diverse environments. However, a fundamental question remains regarding the extent to which the cognitive processes underlying social learning are universal across human societies versus being significantly shaped by cultural context. Much research in behavioral sciences relies on WEIRD (Western, Educated, Industrialized, Rich, Democratic) populations, potentially obscuring cultural variation and limiting the generalizability of findings about learning, innovation, and adaptation. Experimental studies directly comparing the dynamics of cultural evolution across diverse cultural settings are crucial for testing hypotheses about universality and understanding the interplay between cognition and culture.

Materials and methods: We employed a transmission chain experiment involving iterative 3D artifact design in a 5-dimensional parameter space. Each isolated transmission chain included three generations. Participants were recruited from three distinct populations chosen to span cultural and socioeconomic diversity: Prague, Czech Republic (WEIRD university students); Buea, Cameroon (non-WEIRD university students); and Bokwango, Cameroon (non-WEIRD rural village). Participants were instructed to use a custom 3D object configurator to design an artifact optimized for a given task. Within each population, participants engaged in either an Engineering task (minimize rolling time of a 3D printed version down a sloped ramp), an Aesthetics task (maximize peer-rated visual appeal of a 3D printed version), or an Averaging task (estimating the mean of parent artifacts, serving as a baseline). Participants of the first generation were not shown any artifacts, however, participants in the subsequent generations were provided two or three artifacts from the previous generation for inspiration. We tracked the artifact parameters, 3D printed the artifact, and measured its performance metrics (rolling time, aesthetic ratings) for artifacts across three generations within each chain.

Bayesian hierarchical models are used to compare key micro-evolutionary dynamics - including rates

of performance improvement, trajectories of parameter change, and patterns of within-chain variance - across the populations, and tasks.

Results: Our analyses compare the micro-evolutionary dynamics of artifact evolution across the three cultural settings, aiming to identify universal patterns and culturally specific modulations. Preliminary findings suggest a complex interplay between universal cognitive mechanisms, cultural context, and task type. While some basic learning patterns may be shared, significant population-level differences in adaptive dynamics emerged. Unexpectedly, the Engineering task revealed marked variation linked to educational context within Cameroon; Buea university students showed substantially less performance improvement across generations compared to both Prague students and, notably, the Bokwango rural participants. This suggests that local educational environments might interact with cultural learning styles, potentially influencing strategies like exploration versus conformity, with downstream consequences for adaptation even in objective, functionally constrained tasks. Conversely, the Aesthetics task may show different patterns of cross-group variation, possibly reflecting distinct local norms or evaluation criteria. Complete quantitative results will be presented at the conference.

Bayesian model comparisons will quantify the evidence for these population differences, task interactions, and variations in specific dynamics across groups.

Conclusions: This research provides experimental investigation of the universality and cultural specificity of micro-evolutionary dynamics underlying artifact evolution. The findings highlight how both shared human cognitive capacities and diverse cultural backgrounds shape processes of adaptation, innovation, and transmission. By moving beyond WEIRD samples, this study contributes to a more robust understanding of cultural evolution, the prerequisites for cumulative culture, and the interplay between cognitive constraints and cultural learning strategies.



Unraveling the gene regulatory networks of predator-induced developmental plasticity within and across generations in *Daphnia magna*

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POSTER SESSION 1 - **P01.252**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

The dynamic interplay between genetic and environmental factors has long captivated biologists aiming to understand phenotypic variation across environments. Developmental plasticity is a remarkable feature where a genotype may display multiple phenotypes in response to various environmental factors, without changes to the DNA sequence itself. This plasticity can manifest within a single generation (within-generation plasticity, WGP) or across generations (transgenerational plasticity, TGP). *Daphnia magna*, known for its well-documented responses to environmental changes and predation—including alterations in morphology, behavior, and life-history traits—and its asexual reproduction, serves as an ideal model for studying these phenomena. Recent research has revealed gene expression changes during plastic responses, yet the gene regulatory mechanisms driving WGP and TGP remain largely unexplored. By combining traditional gene expression assays (RNA-seq) with

Assays for Transposase-Accessible Chromatin sequencing (ATAC-seq), this project will investigate the molecular mechanisms that control plastic responses to predator cues. Specifically, the project aims to (1) characterize regulatory mechanisms involved in WGP, (2) examine their persistence across generations in TGP, (3) explore small RNAs' role in epigenetic transmission, and (4) compare the underlying mechanisms of WGP and TGP. By leveraging *Daphnia*'s clonal reproduction and short generational cycles and excellent standing as an ecological model, this study seeks to provide new insights into the epigenetic foundations of plastic development. My poster will share preliminary data on the phenotypic responses of *Daphnia* to predator cues, with a focus on morphological and life-history trait variations across generations. Additionally, I will present initial gene expression data to investigate the molecular basis of these responses.



Unraveling the migratory puzzle: population genetics of the least flycatcher (*Empidonax minimus*)

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POSTER SESSION 1 - P01.245, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Over the last six decades, avian aerial insectivores in North America have experienced overall ~60% population declines across their breeding ranges. Understanding their population genetic structure and spatial connections across the annual cycle is critical to determining potential factors driving these trends; however, this information is limited for many species. We will use genomics (RADseq) to study population structure and connectivity in least flycatchers (*Empidonax minimus*) across North America. Through field and museum sampling, we collected blood, feather, and/or tissue samples from 22 sites throughout the breeding range and during migration in Canada and the USA, and on the non-breeding grounds in Mexico. We will use principal coordinates analysis (PCoA), STRUCTURE, and Least Cost Path analyses to define population genetic structure and

determine how landscape features impact connectivity. We will also analyze samples from individuals captured during migration and on their non-breeding grounds to assess the migratory connectivity of populations over the annual cycle. Preliminary results from a PCoA show some differentiation in genetic structure between Alberta and British Columbia samples suggesting that the Rocky Mountains potentially represent a migratory divide. Birds from the non-breeding grounds clustered with the Alberta population. Future analyses with a larger sample size will further refine population connectivity for this species. This research will increase knowledge of the least flycatcher population connectivity between breeding, migration, and non-breeding sites and estimate levels of genetic differentiation among populations.



Urban warriors have bigger shields – how urbanization affects ornament expression and individual quality in a common waterbird

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POSTER SESSION 1 - P01.020, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Expression of exaggerated sexual traits (i.e. ornaments) may be associated with different fitness-related traits (such as reproduction or survival), as it signals individual quality and dominance. However, ornaments also allow to reliably assess quality of potential competitors or mates. Therefore, variation in the expression of ornaments may be shaped by either natural or sexual selection. Visual components of sexual communication are also affected by environmental conditions, which may be heavily altered in human-dominated ecosystems. A wide range of urban ecology studies has shown differences in ornament expression between urban and nonurban bird populations. However, most of them focus on carotenoid-based plumage, while the information about variation in the expression of bare (non-plumage) and colorless ornaments is scarce. Here, we assessed the associations between urbanization and the expression of purely white bare ornament (i.e. frontal shield) in a common waterbird, the Eurasian coot *Fulica atra*.

Methods: During long-term monitoring, we quantified the size of the frontal shield in ca. 480 adult Eurasian coots from four urban and four nonurban populations across Poland. Next, we tested for associations between ornament expression and urbanization, body condition (haemoglobin concentration and

size-corrected body mass), physiological stress (heterophil/lymphocyte ratio), nest site characteristics, nest defence, and reproductive performance of coots.

Results: In our study, urbanization positively affected the ornament expression, as urban coots had bigger shields than nonurban ones. Ornament expression was also positively associated with hemoglobin concentration, but not with size-corrected body mass and physiological stress. We found relationship between the shield size and nest site selection pattern, as coots with bigger shields built nests more exposed to human-related stressors. Finally, we found no evidence for associations between shield size and either nest defence behaviour or reproductive success of coots.

Conclusions: To our best knowledge, our study provided the first evidence for the positive effect of urbanization on the size of the bare colorless ornament in birds. Our results also show the condition-dependent character of ornament expression in the Eurasian coot, which suggests that the shield size may be considered as the honest signal of individual quality. Our results clearly indicate that urbanization can modulate the processes of competitor assessment and mate selection in birds, suggesting that the regimes of sexual selection may change across urbanization gradients.



Using a computational model to explore possible eco-evolutionary drivers of swarming behavior in locusts

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POSTER SESSION 3 - P03.300, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The phenomenon of gregarious locust swarms is a unique occurrence in which grasshoppers form massive, coordinated swarms comprising vast numbers of individuals that travel great distances. This phenomenon has garnered global attention due to its devastating impact on agriculture, threatening the food security of millions of people. The majority of research on this phenomenon has focused on its mitigation, emphasizing the life history traits and biology of locusts. However, the eco-evolutionary underpinnings of locust swarming remain underexplored.

In this study, I utilize an agent-based computational simulation to investigate how the ecosystem and population dynamics of locusts determine the profitability of large-scale swarming behaviour. Most locust

species inhabit arid or semi-arid regions, and may suffer from local extinction events of populations within the broader spatial landscape. The hypothesis I explore is that the potential for recolonization via long-distance travel of locust swarms confers an evolutionary advantage that led to the development and retention of this extreme dispersal strategy. This advantage may promote the persistence and adaptive success of such swarming behaviour. Using spatially explicit simulations allows me to explore the role of factors such as environmental variation and its spatio-temporal autocorrelation, population dynamics, and patch distribution, as well as their interaction, as the evolutionary drivers of locust swarming.



Using ddRADseq to explore the genomic landscape of serpentine habitation in three diploid plant species from the Iberian Peninsula

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POSTER SESSION 1 - P01.086, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Background: Serpentine soils constitute an extreme environment for plant survival, characterized by a combination of chemical properties that include low levels of essential nutrients and high concentrations of toxic heavy metals. These harsh conditions exert strong selective pressures, limiting the diversity of flora that can establish and persist. Despite these challenges, some plant species have developed specific physiological, functional, and genetic mechanisms that allow them to thrive in serpentine habitats. Understanding the genomic basis of serpentine adaptation provides valuable insight into evolutionary processes, local adaptation, and biodiversity conservation in edaphically stressful environments. This study focuses on three diploid species native to southern Iberian Peninsula - *Lavandula stoechas* L., *Halimium atriplicifolium* (Lam) Spach. subsp. *atriplicifolium*, and *Phlomis purpurea* L.- and aims to investigate the extent and nature of genomic differentiation associated with serpentine versus non-serpentine soils.

Materials and Methods: We employed double-digest Restriction-site Associated DNA Sequencing (ddRADseq) for generating genome-wide SNP datasets in non-model organisms, aiming to explore patterns of genomic differentiation. For each of the three focal species, we sampled 20 populations, equally divided between serpentine and adjacent non-serpentine soils, forming 10 geographically paired serpentine/non-serpentine population sets, located in the largest serpentine outcrop in the Iberian Peninsula. The STACKS bioinformatics pipeline was used to process raw sequencing data and construct species-specific SNP datasets. We used multivariate genotype ordination approaches, including Principal Component Analysis (PCA) and Multidimensional Scaling (MDS), to visualize patterns of genomic variation. Additionally, we performed admixture analysis to estimate the number of ancestral

genetic clusters and assess the extent of shared ancestry among populations on different soil types. Treemix analysis was conducted to infer historical patterns of population divergence and detect possible gene flow.

Results: The genomic analyses reveal distinct, species-specific histories of the colonization of serpentine environments. In some cases, serpentine and non-serpentine populations form well-differentiated genetic clusters, indicating reduced gene flow and the potential for local adaptation, particularly in the Sierra Bermeja area (Málaga). In other species, we observe more complex patterns, including signs of admixture and evidence for multiple independent diversification events into serpentine habitats. These findings suggest that the evolutionary pathways to serpentine colonization are not uniform across taxa but may instead reflect a combination of historical divergence, ongoing gene flow, and potential for local selection pressure. Furthermore, the Treemix results provide insight into the dynamic interplay between isolation and connectivity among populations across a diverse edaphic landscape.

Conclusions: This study contributes to a deeper understanding of how plants can inhabit extreme edaphic conditions, such as those presented by serpentine soils. By integrating genome-wide SNP data with landscape-level population sampling, our data suggest that habitation of serpentine environments in southern Iberian Peninsula involves historical and potentially contemporary evolutionary processes. The species-specific nature of genomic differentiation underscores the complexity of serpentine colonization and highlights the need for comparative studies across multiple taxa. Our results lay the groundwork for future research into the specific loci involved in adaptation and have potential applications in the conservation of plant species in environmentally fragile and unusual habitats.



Using drawings to illustrate evolution

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POSTER SESSION 3 - P03.322, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

The key ingredients of Darwinian evolution are heredity, differential fitness and variation. As long these components apply to any system, even non-biological systems, evolution occurs. An example of evolution in a non-biological system investigated is the evolution of drawings.

When a picture is redrawn by different people, the new set of drawings resemble (inherit) aspects of the first drawing but also show new variations. When one of these new drawings is selected based on a specific characteristic and again redrawn by different people, repetition of this process will gradually change the drawing's characteristic(s) under selection.

This system proved to be an interactive and highly useful educational method to illustrate important evolutionary concepts

such as variation, heritability and differential fitness, but also more complex evolutionary principles like effects of population size, trade-offs, phylogenetic reconstruction.

Despite the absence of a clear genetic structure in this system, even heritability estimates can be performed. The high resemblance of this system with organismal evolution makes it an interesting exercise that shows how Darwinian laws (i.e. descent with modification) can be applied to other substrates. By comparing this system to biological evolution might give more insight on whether there is a fundamental difference between the definition of biological evolution (i.e. change of genetic composition in a population) and the more classic Darwinian definition (i.e. descent with modification).



Using moulting cycle time series transcriptome analysis to pinpoint host candidate genes for the *Daphnia magna* – *Pasteuria ramosa* host-parasite interaction.

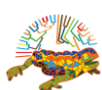
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POSTER SESSION 1 - **P01.234**, EXPO AREA, August 18, 2025, 5:00 PM - 7:00 PM

Coevolution has been suggested to be a major driver of biodiversity, shaping genetic and phenotypic evolution in natural populations. *Daphnia magna* and the bacteria *Pasteuria ramosa* have been coevolving for at least 15 million years. Different *D. magna* strains can be either susceptible or resistant to a given strains of *P. ramosa*. Genomic studies have identified genomic regions enriched for glycosyltransferases, but strong linkage prevented to pinpoint the very gene that is responsible for resistance. Parasite attachment is believed to be driven by the interaction between the proteins present on the surface of the parasite and the sugars attached to the glycoproteins on the cuticle of the host. We speculate that these sugars are expressed during the formation of the

new cuticle before moulting. To get a better understanding of the main actors of this interaction, we conducted a time series transcriptomic study in *D. magna* across the entire moulting cycle. This study will help us to identify if and when glycosyltransferases are expressed during building of the new cuticle, and with which proteins they are co-expressed. By this, we might eventually reduce the candidate genes involved in the attachment. From this work, we will be able to select candidate genes that we will knockout/knockdown, to investigate their effect on parasite attachment. Moreover, the dataset we will obtain will be extremely useful for future studies as a background of how different gene families behave during the moulting cycle of *D. magna*.



Vocal divergence and incipient speciation in the Reunion Grey White-eye

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POSTER SESSION 3 - P03.270, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Understanding how small and structured populations diverge is key to revealing the mechanisms of speciation. On islands, where populations are often small, evolutionary processes such as drift, local adaptation, and cultural transmission can drive rapid divergence. In songbirds, vocalisations play a central role in individual recognition and social communication, and rapid cultural evolution in geographically separated populations can be accompanied by song divergence, which may contribute to the evolution of behavioural isolation. Thus, vocalisations in songbirds can play a role in restricting gene flow hence contributing to speciation. In this study, we characterised the vocalisations of the Reunion Grey White-eye (*Zosterops borbonicus*), a clear case of recent lineage formation, which comprises four distinct plumage forms (three lowland and one highland form). It is hypothesised that the highland form likely emerged through local adaptation to the dense cloud forests and subalpine scrubland of the high altitudes, while the lowland forms likely diverged through sexual selection. We investigated vocal divergence among the four forms, focusing on song composition, syllable diversity, and shared syllables

across forms. Additionally, we assessed whether acoustic differentiation is associated with geographic distance, genetic differentiation, or both, while controlling for form identity. We analysed songs from individuals distributed across 11 different localities in Reunion by using novel approaches based on machine-learning algorithms. Trained algorithms could reliably distinguish populations based on syllable use and selectivity, and acoustic features, suggesting detectable structure despite overlapping syllable types. Acoustic distances between forms were generally higher than those observed within forms, but this trend lacked statistical significance. Similarly, acoustic distance between lowland forms was not significantly higher than between highland and lowland forms. Moreover, neither geographic nor genetic distances were correlated with acoustic variation among populations. Our findings highlight potential complex dynamics involving cultural transmission and social interactions. Exploring calls and song syntax may provide deeper insights into communication and reproductive barriers, enhancing our understanding of the evolutionary processes.



White lupin's genomic diversity and domestication history through pangenome graphs

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POSTER SESSION 3 - P03.279, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Domestication is a human-mediated process promoted by the transition from a hunter-gatherer subsistence economy to a farmer-oriented one. In the white lupin (*Lupinus albus*), such a process was potentially initiated in the Old World by ancient Greeks; from then onwards, different varieties of this species have been gradually and locally selected across the Mediterranean basin. However, the origins and whether domestication involved a single or multiple events remain unclear. Furthermore, genomic structural variants (SV) are recognized for their role in domestication, contributing to local adaptations to diverse environmental conditions in many species. Established bioinformatics approaches combining short reads and linear reference genomes are effective in SNP and INDEL discovery, but they systematically fail to capture the full spectrum of SV, and, consequently, the impact of SV on domestication is often understudied.

To overcome these limitations and investigate the genomic diversity underlying

white lupin domestication, we combined Pacbio HiFi reads and Hi-C data to construct a chromosome-scale pangenome graph incorporating 16 phased genome assemblies of both wild and landrace accessions from across the Mediterranean basin. A pangenome graph is recognized as a better model for encapsulating the full spectrum of variants along multiple whole genomes, reducing or preventing reference bias and providing a more comprehensive representation of the species' genetic diversity. The pangenome graph was then integrated with more than 300 short-read whole-genome resequenced accession datasets to characterize genomic variation, reconstruct the domestication history of white lupin, and identify key genomic regions targeted by artificial selection. Our pangenome-based approach offers novel insights into the complex genomic changes associated with white lupin domestication and highlight the contribution of SVs in this process.



Would the real *Ophioplocus incipiens* please stand up?

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POSTER SESSION 3 - **P03.271**, EXPO AREA, August 21, 2025, 5:00 PM - 7:00 PM

Background: Ophiuroids are a diverse echinoderm group in the Southern Ocean. However, current records are likely to represent only a fraction of the true species richness as taxonomic complexity complicates comprehensive assessments. In some species taxonomic characters are highly variable within the same species and shared between different species, which can mislead experts in correct identification. In others there are no distinguishing characters between genetically and geographically isolated species. Here we examine the distribution and genetic patterns of *Ophioplocus incipiens*. This ophiuroid has an interesting distribution with verified specimens from South Georgia shelf, South Orkney Islands shelf, Elephant Island surrounds, King George Island (South Shetland Islands), the Eastern Weddell Sea and the East Antarctic shelf near Dumont d'Urville station. Given its broad latitudinal range, its apparent absence from the Antarctic Peninsula, Bouvetøya and other Southern Ocean islands is curious. Where it is located, it is found in great numbers. These observations raise the biological question of whether the species exhibits underlying genetic differences indicative of cryptic diversity and a more complex population structure. Here we test the hypothesis that *O. incipiens* is a single, panmictic species across its range, through phylogenetic reconstruction and haplotype networks.

Materials and methods: Sampling was conducted on several cruises using trawls at various depths on the continental shelf and slope around the Antarctic continent and Southern Ocean island shelves. Formal identification involved sorting of specimens into morphotypes, followed by more detailed comparisons using literature descriptions

and comparing with images of syntypes. Sequences were obtained from the mitochondrial Cytochrome c Oxidase subunit 1 gene (mtCO1), nuclear ribosomal 18S (small subunit) and 28S (large subunit) genes.

Results: MtCO1 sequences show deep divergences between the South Georgia samples and those from South Orkney Islands and the Antarctic shelf. Nucleotide diversity was very high in the South Orkney Island and King George Island populations, with some shared haplotypes. The Weddell Sea samples failed to amplify indicating a mutation in the primer binding site, suggesting a difference from the other samples. Redesigned primers and PCR optimisation are underway to better understand if there were a specific population structure. Nuclear markers are being used to gain independent support for the patterns being uncovered with the mitochondrial marker.

Conclusions: This study suggests that *Ophioplocus incipiens* is, like other Southern Ocean ophiuroids, a complex of several species. The South Orkney Islands and the South Shetland Islands have complex, diverged lineages, previously observed in *Amphiura belgicae* from those regions. It has been suggested that the observed radiations in ophiuroid species had a common cause, suggesting that all members of Southern Ocean assemblages were subject to a shared history and may exhibit the same cryptic diversity patterns.





THANK YOU