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Evoluzione

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Book of Abstracts



OPENING LECTURE

Evolution through trait loss

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Loss of metabolic function is a classic hallmark of symbiotic interactions. When a symbiotic partner reliably provides specific nutrients or functions, the receiving organism often becomes dependent, a transition frequently accompanied by the decay of the underlying biosynthetic genes. Insect parasitoids, which lay their eggs inside or on other insects, rely entirely on their hosts to complete development and throughout evolution have repeatedly lost the ability to accumulate lipids. However, the genetic machinery encoding lipid synthesis has remained intact, suggesting regulatory changes underlie phenotypic loss, possibly driven by pleiotropic effects of lipogenic genes. Through comparative and experimental studies, I will explore the genetic architectures that shape evolutionary trajectories when a trait is no longer under direct selection. Combining RNAi knockdown experiments with tissue-specific expression analyses and quantitative measurements of lipid-related phenotypes, I will unravel the role of pleiotropy during phenotypic loss. Furthermore, our findings reveal an intricate web of regulatory rewiring within the underlying gene networks. Ultimately, investigating the loss of lipid accumulation challenges the traditional view of evolution as a unidirectional path toward increasing complexity, offering key insights into the constraints and potential reversibility of symbiotic relationships.

CAVALLI-SFORZA LECTURE

From evolutionary genetics to practical benefit: Ideas, tools, and action to bolster coral reefs against heat waves

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Natural populations across all ecosystems face challenges in adapting to current and future environmental conditions, and so processes and rates of adaptation are key elements for future sustainability. New population genomics tools that link genotypes with phenotypes allow us to explore the links between polymorphisms and fitness in different environments. In ocean settings we have shown links between gene polymorphisms and growth under different temperature and acidification conditions for sea urchins, Mediterranean corals, abalone, and Pacific reef corals. More recently, we are focusing on genome-level adaptation within and between species of corals, and their symbionts, with the specific goal of understanding the adaptive landscape of adaptation to high temperatures. Yet, while we are exploring the mechanisms of heat resistance in corals, we have an opportunity to use the phenotype of heat resistance as a tool to help rebuild reefs with heat tolerant individuals of many species. By simplifying testing for heat resistant phenotypes we have opened this option for reef restoration projects in many coral reef settings, especially in the island Pacific. Cycling back to the basic science, these nurseries of high resistance corals open opportunities for field tests of genetic tools, breeding programs and symbiosis resilience.



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TALKS



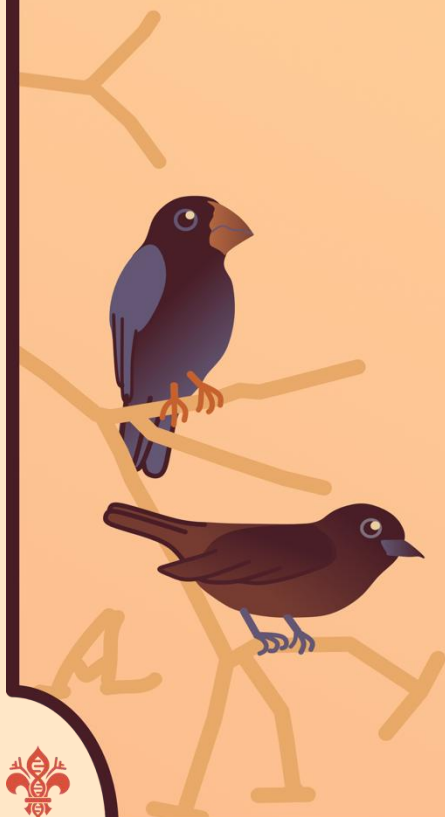


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Symposium 1: “Host-microbiome interactions”

Chairs: Giovanni Bacci, Camilla Fagorzi



Invited lecture

Trained immunity in host–microbiome interactions: an evolutionary perspective

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Immunological memory is an evolutionary trait which provides clear advantages for a host, yet it was hypothesized that it can be induced only in the presence of an adaptive immune response, hence in vertebrates. Recent work has however demonstrated that immune memory characteristics based on epigenetic reprogramming of innate immune responses (trained immunity) are present in plants, invertebrates and vertebrate animals, implying a much earlier evolutionary origin than adaptive immune memory. Induction of trained immunity is mediated by activation of immune and metabolic pathways that result in epigenetic rewiring of cellular functional programs. Chromatin accessibility as assessed by histone modifications at the level of enhancers is an important mechanism mediating these programs. The role of trained immunity in host defense against pathogens, as well as in immune-mediated diseases, will be discussed.

Unveiling the microbiome biodiversity and biogeography of spontaneous wine fermentations through shotgun metagenomics

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Modern winemakers commonly inoculate grape musts with selected *Saccharomyces cerevisiae* strains to obtain controlled and reproducible products. However, traditional spontaneous fermentations (SP), where fermentation is driven by indigenous microbiota, are regaining interest among small producers. While amplicon-based surveys have revealed biogeographical patterns in wine microbiota, these approaches offer limited taxonomic and functional resolution, and much remains unknown about the ecology, evolution and functional genomics of wine microbiomes. Shotgun metagenomics can extend the microbial terroir concept beyond species composition to functionally distinct strains that shape regional wine characteristics. Yet, so far, shotgun metagenomics evidence is still limited to a small number of single-country studies. Here, we investigate the microbial biodiversity of 59 spontaneous fermentation must metagenomes from 21 Italian estates and compare them with publicly available shotgun datasets from Germany, Spain and Australia. We reconstructed MAGs for functional analyses, inferred strain-level phylogeny of *S. cerevisiae*, and used a curated wine-specific Kraken database for taxonomic profiling, overcoming the under-representation of oenological taxa in reference databases. We found clear country-level biogeographical structure, with a small global core microbiome within a large variable pan-microbiome. The *S. cerevisiae* phylogeny placed most SP strains within the wine clade and revealed no clear geographic structuring between spontaneous and commercial strains. We are currently testing the stability of the microbial community over time and the ecological origin of *S. cerevisiae* strains (grapevine versus cellar) by analysing multiple time points and

microvinified samples. Together, our results support a microbial terroir of spontaneous wine microbiomes, while *S. cerevisiae* populations remain genetically homogeneous within the wine clade.

They are what they eat - effects of diet on the gut microbiota of the gray wolf (*Canis lupus*)

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The grey wolf (*Canis lupus* L.) is undergoing a notable increase in population size and range across Europe, and the recolonization of peri-urban and agricultural areas may lead to dietary changes due to increased predation on livestock. While well-studied in humans, the effect of dietary changes on the gut microbiota of wildlife in human-impacted environments remains largely unknown. Here, we investigated dietary impacts on wolf gut microbiota by analysing faecal samples collected with non-invasive methods from a pack of nine healthy wolves hosted by the Belpark – Parco Faunistico Spormaggiore (Italy). Over a three-month period, wolves were sequentially fed a diet based on either whole sheep (*Ovis aries*) or roadkill red deer (*Cervus elephas*) before returning to a conventional mixed diet. Prokaryotic and fungal communities the entire pack throughout the experiment were characterized using metataxonomy (16S rRNA V3-V4 and ITS2 gene regions). To link taxonomic changes to changes in gut function, the gut microbial transcriptional profile of a subset of 20 samples was investigated using metatranscriptomics. Longitudinal comparisons revealed a small core microbiota of dominant bacteria and fungi shared by all animals, numerous low-abundance or individual-specific microorganisms. Shifting to a wildlife-based diet elicited contrasting responses: bacterial richness increased rapidly but transiently, whereas fungal communities exhibited a continuous increase in richness and greater inter-individual variability. Fungal community composition was strongly associated with diet, while bacterial composition was only marginally linked. Our results clarify the temporal natural variation of the gut microbiota in captive, related animals sharing a controlled home range. These findings have direct implications for wildlife health, contributing to dietary regimes that preserve microbial diversity and functional potential, thereby supporting animal welfare in wildlife parks.

Rules of attraction: *Saccharomyces cerevisiae* volatile metabolite profiles mediate insect–yeast associations

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The dispersal, mating, and evolution of natural *Saccharomyces cerevisiae* populations are significantly driven by social wasps, which represent a key reservoir for this yeast. While the role of these insects as hosts is well-documented, the mechanisms driving the selection of specific yeast strains during the uptake remain largely unknown. Yeast-derived Volatile Organic Compounds (VOCs) could act as chemical cues, potentially resulting in the preferential dispersal of strains based on their ecological adaptation to insect-associated niches. Here, we investigated how VOCs produced by *S. cerevisiae* strains from different natural sources shape social insect attraction by simulating floral nectar, focusing on the branched-chain amino acid aminotransferase gene BAT2, which acts upstream of the Ehrlich pathway. To evaluate this, selected strains and their specific VOCs—identified and quantified via Gas Chromatography-Mass Spectrometry (GC–MS)—were tested for their ability to attract *Polistes dominula* and *Apis mellifera*. Behavioral assays demonstrated species-specific responses, highlighting differential ecological effects. To validate the role of fusel alcohols in attraction, we performed a deletion of the BAT2 gene in the *Polistes dominula*-attractive wasp-gut isolated strain, yielding monoallelic and null mutants of the BAT2 gene whose VOC profiles were analysed using GC–MS. This gene deletion resulted in significant metabolic shifts, including altered production of branched-chain alcohols and acetic acid, which mediate insect behavioral responses. The null mutant showed the most pronounced variations in VOC emissions compared to both wild-type and monoallelic strains. These findings demonstrate that BAT2 shapes yeast volatile profiles by modulating key attractive compounds. Our results

shed light on how VOCs mediate the attraction of different social insects, contributing to our understanding of yeast ecology and vector interactions.

Microbiome Strain Sharing and Co-diversification across Sympatric Wild Rodents Species

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Mammalian gut microbiomes are shaped by both host evolutionary history and shared environments. However, these factors are often confounded in experimental designs, making it difficult to disentangle their relative contributions to microbiome variation. Here, we used full-length 16S rRNA sequencing to obtain strain-level information on gut microbiomes from 524 individuals across 15 rodent species inhabiting two Madrean Sky Islands in southern Arizona (USA)—isolated mountain ranges separated by desert lowlands that function as replicated natural ecosystems. In this system, closely and distantly related species coexist with varying degrees of sympatry, allowing us to test how evolutionary and ecological processes influence microbial strain sharing and host–microbial co-diversification. We found that host phylogeny emerged as the primary predictor of bacterial strain sharing across species, exceeding the effects of habitat similarity and geographic proximity. We identified specific bacterial clades showing strong signatures of host–microbial co-diversification across the bacterial phylogeny, demonstrating that long-term evolutionary coupling between hosts and microbes is widespread even within sympatric mammal communities. Furthermore, these patterns were linked to bacterial life history traits, with oxygen sensitivity emerging as the strongest predictor of host-to-host transmission. While aerotolerant bacteria exhibited broader host ranges and higher rates of interspecific sharing, co-diversifying taxa were predominantly obligate anaerobes with restricted host ranges, suggesting that dispersal limitation promotes long-term host associations. Our results demonstrate that host evolutionary history best predicts gut microbiome composition and strain sharing, and atmospheric oxygen is a barrier that reinforces dispersal limitation and promotes stable host–microbe symbiosis across evolutionary timescales.

Genomic and transcriptomic analysis of the host-coronavirus interactions

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Seven different coronaviruses infect humans, including four causing the common cold and three causing more severe respiratory infections. Because these coronaviruses all use human translation machinery to translate their mRNAs, they have evolved various ways of adapting to the host translation machinery. To make a virion, some viral proteins (e.g., nucleocapsid protein) are needed in large numbers, whereas some other proteins are needed only in a few copies. This implies the selection pressure for more efficient protein production for some proteins than for others. Protein production increases with translation initiation, elongation and termination efficiencies as well as mRNA abundance (which in turn depends on mRNA production and degradation). We perform 1) genomic analysis to dissect how mutation and natural selection shape the translation initiation region, the codon optimization and stop signal usage in coronavirus genes, 2) transcriptomic analysis of viral mRNA abundance during viral infection cycles (which is complicated by the special way of viral subgenomic mRNA production through transcription regulatory sequences), and 3) transcriptomic characterization of host immune response genes (e.g., antiviral proteins), with specific emphasis on how viral patterns are recognized and responded to. Different lineages of coronaviruses adapted to human hosts in their respective ways, resulting in different patterns of host-pathogen coexistence.

Evaluating habitat health through species-level diet-microbiome interactions in an alpine specialist

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Understanding habitat health is fundamental for wildlife conservation. Fecal samples represent a non-invasive source of ecological information, yet their potential to simultaneously inform on diet, animal condition, and environmental quality remains largely unexplored. Here, we show how an advanced shotgun metagenomics framework overcomes the taxonomic limitations of standard methods, resolving dietary and microbial profiles at the species level. This allows us to explore how dietary-driven microbial taxa can serve as robust indicators for habitat health. Focusing on the Alpine rock ptarmigan (*Lagopus muta helvetica*), a boreal, cold-adapted glacial relict inhabiting high-altitude environments in the Italian Alps, we evaluated the performance of shotgun sequencing and Kraken2-based taxonomic annotation across diverse, quality-controlled databases of full genomes and marker sequences. Our co-occurrence network analysis reveals ecology-driven associations between plant and bacterial taxa, with specific gut bacteria emerging as indicators of habitat quality. *Pseudomonas putida*, a species associated with stressed soils, positively correlates with the habitat showing the lowest plant diversity, while *Methylobacterium bullatum* identifies habitats of high ecological quality. We also find that specific dietary taxa directly indicate animal health, as shown by the negative association between medicinal plants such as *Lavandula angustifolia* and potentially pathogenic bacteria. Additionally, the detection of plant pathogens like *Rhizobium tumorigenes* across sampling sites demonstrates that a single fecal sample simultaneously captures host condition, diet, and plant-ecosystem integrity. These findings demonstrate that integrated diet-microbiome analysis of fecal samples provides a multi-layered, non-invasive, and transferable tool for assessing habitat health, offering wildlife managers a new approach to monitoring ecosystem condition through host diet-microbiome interactions.

Ecology and evolution of the wine fermentation microbiome revealed by shotgun metagenomics

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Fermented food environments are compelling systems for studying host-microbiome interactions, as microbial communities are shaped by substrate, geographic origin, and long-term co-evolution with human practices. Resolving their taxonomic and functional structure requires culture-independent approaches that go beyond classical amplicon sequencing. We developed a shotgun metagenomic pipeline optimised for non-model matrices, using wine fermentations as a case study. It integrates custom Kraken reference database curation for species-level community profiling, Metagenome-Assembled Genome (MAG) reconstruction, and strain-level phylogenetic inference, enabling accurate resolution of *Saccharomyces cerevisiae* diversity directly from environmental samples. Applying this framework alongside global food and human gut metagenomes revealed that *S. cerevisiae* strains in fermented foods span multiple domesticated lineages, with human-associated strains clustering at the base of the Liquid State Fermentation superclade. This pattern supports the human-gut domestication hypothesis, suggesting ancient intra-gut domestication of yeast predating deliberate fermentation use. Systematic screening of publicly available metagenomes yielded a phylogeny of over 13,000 *S. cerevisiae* strains, the largest reconstructed to date, identifying novel source-specific lineages and challenging assumptions about geographic structuring within the wine clade. These findings establish fermentation microbiomes as tractable models for host-microbiome co-evolution, and the human-gut domestication hypothesis opens new perspectives on how microbial associations with human hosts may have driven the diversification of one of the most widely used organisms in food biotechnology.

De novo assembly of ancient metagenomic data from dental calculus to disentangle the Neolithic transition in Southern Europe

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The interest in ancient metagenomics is increasing with over 1.700 ancient microbial genomes, partial genomes, and metagenomes characterized. However, most studies focused on aligning sequences to modern reference genomes, limiting discoveries to known taxa and introducing a reference bias, as ancient and modern genomes differ genetically. A way to overcome this is through de novo assembly, which reconstructs genomes of all species present in a sample. We evaluated multiple de novo assembly pipelines using simulated ancient metagenomes and identified the optimal pipeline for reconstructing ancient metagenome-assembled genomes. We applied this approach to ancient metagenomes covering the Neolithic transition, a major cultural and demographic event in Europe that affected both human evolution and the oral microbiome. We collected published metagenomic data from different regions in Europe ranging from the Palaeolithic to the Bronze Age, along with newly sequenced samples from Italy. We focus on reconstructing the ancient genome of *Anaerolineaceae* oral taxon 439, a bacterium found at higher abundance in the oral microbiome of Neolithic farmers. Through genome reconstruction, we investigated its genetic variability and perform functional annotation to identify genes that may have been lost/gained and can have an impact in the metabolism of food/medicines.

There is one impostor among us: species boundaries, genomic divergence, and Wolbachia absence in the cryptic species *Culex juppi*

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The *Culex pipiens* complex, a worldwide distributed group of mosquitoes and the primary vector of West Nile virus in Europe, represents a paradigm in evolutionary biology and molecular systematics. This complex comprises an intricate mosaic of morphologically indistinguishable lineages that, despite overlapping cosmopolitan ranges, exhibit profound ecological and genetic divergences. In this context, the identification of the cryptic species *Culex juppi*, a recently described taxon characterized by the absence of the *Wolbachia* endosymbiont, has raised new questions about the evolutionary dynamics governing cryptic speciation within the group. Utilizing an integrated approach that combines population genomics via double-digest restriction-site associated DNA sequencing (ddRAD-seq), *Wolbachia* molecular screening, and mitochondrial DNA analysis, we investigated phylogeny, population structure and genetic divergence of *Cx. pipiens* and *Cx. juppi* populations in a newly identified area of sympatry in the Emilia-Romagna region (Italy). Moreover, we characterized the cryptic species *Cx. juppi* at the genomic level, with particular attention to the polymorphisms associated with the *Wolbachia*-free phenotype. Overall, these findings not only expand the current knowledge about cryptic speciation within the *Culex pipiens* complex, but could also contribute to improving vector control management for public health.

The case of *Helicobacter pylori*: the consequences of the alteration of an internal ecosystem

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When *Helicobacter pylori* was identified as living in the stomach mucosa, its presence was proved to be an infection that caused gastritis, gastric ulcers, and cancer. As a result, the bacterium was classified as a certainly harmful species and its eradication became a mandatory therapeutic indication. Subsequent studies showed that (i) the eradication of the bacterium was associated with reflux esophagitis and esophageal cancer; and (ii) in African, Indian, Asian, and even European populations of the last century, the presence of the bacterium was not associated with gastritis, gastric ulcers, and cancer; and (iii) the presence of *H. pylori* had some beneficial effects. Further studies revealed that the harmful effects of *H. pylori* do not occur when intestinal worms are present. These data suggest that a simple analysis of the association of *H. pylori* presence with certain pathologies is incomplete and misleading. Conversely, in the interpretation of evolutionary medicine, it is necessary to consider the interactions between *H. pylori* and other components of the human holobiont (i.e., human species, plus the species that coexist with it). An alteration in the ancestral relationships between the components of the human holobiont, for example by elimination of one or more species considered pathogenic, represents a cause of holobiont imbalance with plausible negative effects on the host's health. The eradication of intestinal worms or *H. pylori*, or both, and the concomitant alteration of microbial ecosystems of the human holobiont are a plausible cause of harmful and even lethal diseases that are increasingly widespread in the modern world.



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Symposium 2: “Evolutionary roots of behavioural diversity”

Chairs: Federico Cappa, Alessandro Cini, Lisa Locatello



Invited lecture

Cognitive Neuroscience in Miniature Brains - Long-Term Memory and Higher-Order Learning in Honey Bees

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Despite possessing a brain of only ~1 mm³, honey bees exhibit a remarkably sophisticated behavioral repertoire and can learn and memorize multiple sensory cues associated with flowers. Beyond serving as convenient models for the study of simple associative learning, bees have also emerged as powerful organisms for investigating higher-order learning in both the visual and olfactory domains. Here, I focus on olfactory learning using proboscis extension response (PER) conditioning. Contrary to conclusions drawn from decades of research, we show that protein synthesis–dependent long-term memories can be formed after a single conditioning trial and can be detected as early as 4 h after training. Moving beyond simple associative learning, we investigated nonlinear discriminations, focusing on negative patterning. We demonstrate that bees can solve this complex learning problem and that intact mushroom bodies are required for successful performance. More specifically, we identify a subset of GABAergic feedback neurons within the mushroom bodies that is essential for solving this discrimination. By presenting these findings, I aim to highlight key experimental challenges and outline future directions for investigating the neurobiology of higher-order learning in insects, with the broader goal of identifying fundamental neural architectures underlying cognitive processing.

At the root of cognitive variation: what fish can tell us

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Although studied for more than a century in human psychology, within-species cognitive variation has attracted the attention of evolutionary biologists only recently. Among the taxa investigated, fish are emerging as particularly important: they display most of the cognitive abilities observed in tetrapods, including learning, inhibitory control, and problem solving, while exhibiting substantial interindividual variation and providing highly tractable systems for experimental research. By exploiting this group, we recently investigated the determinants of cognitive variation at both mechanistic and evolutionary levels. In an initial set of studies on zebrafish, chosen for their genetic tractability, we identified a set of genes whose variation predicts individual differences in learning performance. Knockout of the most influential of these genes confirmed the finding, producing a line of fish with impaired learning abilities. Through extended phenotyping of wild and wild-derived species, including tench, eels, and guppies, we further explored the evolutionary mechanisms maintaining cognitive variation. First, we found that individual differences in cognitive traits covary with variation in physiological, behavioral and life-history traits, creating the potential for constraints and indirect selection. Second, these suites of covarying traits predict fitness-related behaviors. Our work on fish reveals previously unknown drivers of cognitive variation and suggests that this group represents a particularly powerful experimental system for addressing fundamental questions about the evolution of cognition.

Bumblebees alter their attentional scope under threat revealing convergent emotional responses

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In humans, emotional states shape cognitive processing: pleasant experiences broaden attention (“seeing the forest”), while unpleasant ones narrow focus (“missing the forest for the trees”). Investigating whether similar emotion-like states exist in deeply divergent, miniature-brained invertebrates is a subject of intense debate. Crucially, determining whether these states are homologous or functionally analogous to those of vertebrates requires objective, non-verbal readouts. Since animals cannot verbalize internal states, tracking how they alter cognitive processes—such as inducing attentional biases—provides a powerful diagnostic tool to infer underlying emotions, though its use in invertebrates remains in its infancy. We assessed the attentional scope of bumblebees using a human-inspired paradigm. Free-flying bees were trained in a Y-maze to discriminate hierarchical visual stimuli (global shapes composed of local elements) and then subjected to a conflict test with swapped cues. Control bees preferred global spatial configurations over local elements. Crucially, bees exposed to a simulated predator attack shifted their preference from global to local information, indicating that the induced negative state narrowed their attentional scope. Mass spectrometry analysis of bee brains revealed that only attacked bees had significantly elevated dopamine levels. Pharmacological manipulations confirmed this neurobiological link: topical dopamine administration to naive bees mimicked the attack-induced local shift, whereas blocking dopamine receptors prior to testing prevented attentional shift in attacked bees. Our findings demonstrate that tracking systematic changes in cognitive processing provides a reliable proxy to infer negative emotional states in miniature brains. This study offers a robust comparative framework to investigate whether the architecture underlying emotions are evolutionarily conserved or represent a case of evolutionary convergence across phyla.

Effects of early-life environmental enrichment on behavioural plasticity in juvenile European lobsters (*Homarus gammarus*)

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Overfishing has reduced European lobster (*Homarus gammarus*) populations, prompting restocking. However, captive rearing can produce morphological and behavioural phenotypes that diverge from wild ones, potentially hindering responses to environmental change. Testing how enrichment affects coping-related responses to disturbance can identify rearing conditions suited to post-release challenges. We transferred 55 captive-reared juveniles, previously kept under enriched or bare conditions, to arenas with shelter and substrate. We assessed proactive habitat modification, responses to daily disruption of self-arranged habitats, activity rhythms across five days, and repeatability of these behaviours. Enriched individuals showed distinct activity rhythms. Latencies to habitat modification were repeatable, suggesting personality traits, with no treatment effects. Substrate exploration was higher in the enriched group; with time it declined in controls but remained stable in enriched individuals. Controls rearranged more substrate more often, but this behaviour increased in enriched individuals after repeated disturbance, suggesting a proactive response. Shelter translocation was unaffected by previous treatment. By revealing coping mechanisms in juvenile lobsters, this study fills a gap in species-specific behavioural knowledge and suggests that enrichment promotes proactive responses to disturbance, informing protocols aimed at increasing resilience and improving restocking.

Alteration of behaviour and gene expression in the Mediterranean green crab (*Carcinus aestuarii*) under intra- and interspecific competition conditions

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Biological invasions are one of the main drivers of global biodiversity loss, and they strongly affect coastal and estuarine habitats. When a non-indigenous species (NIS) becomes invasive it impacts the functioning of the invaded ecosystem and, by introducing a new competitor, disrupts the biotic relationships among native species. Furthermore, alterations on the native species' gene expression due to competitive stress from the invasive species may have deleterious effects on overall fitness and the ability to adapt to environmental changes. On the other hand, native species may adapt their behaviour to the competitor's presence to maximise their fitness and survivorship. We investigated whether and how the behavior of the native Mediterranean green crab (*Carcinus aestuarii*) may be altered by intra-specific competition and by the presence of the invasive Atlantic blue crab (*Callinectes sapidus*). Both species show a high tolerance to abiotic fluctuations, and share an environmental niche. The behavior of green crabs was videorecorded in mesocosms under control, intra- and inter-specific competitive conditions. The time focal crabs spent in various activities was recorded and analysed. Competition was simulated in aquaria divided by a perforated clear screen with water flowing between the two sides. Thus, individuals were able to perceive each other with no direct interactions. Tissue samples from two organs (hepatopancreas and eyestalks) from a subset of green crabs were collected to investigate the transcriptome-wide gene expression responses to competition with high-throughput RNA sequencing. Video analyses show that both competitive conditions influence the behaviour of green crabs in both sexes. Furthermore, the presence of blue crabs alters the behavioural patterns of green crabs more strongly. Transcriptomic analyses show a similar pattern of alteration of gene expression, with interspecific competition affecting green crabs more than the intraspecific one.

Behavioral plasticity of the rocky-shore crab *Pachygrapsus marmoratus* in response to artificial light at night and marine heatwaves

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Despite continued coastal urbanization, little is known about how artificial light at night (ALAN) affects rocky-shore invertebrates and interacts with other stressors such as marine heatwaves (MHWs). In this study, we investigated the effects of ALAN and MHWs on the behavior of the intertidal crab *Pachygrapsus marmoratus*. We hypothesized that ALAN would increase perceived predation risk, causing crabs to both avoid submerged areas at night and reduce their nocturnal activity. We also expected MHWs to both promote emersion, allowing individuals to reduce the metabolic costs of respiration, and increase the nocturnal activity, when air temperatures are lower. Because both stressors were predicted to affect nocturnal behaviours, we further hypothesized additive or synergistic effects under combined exposure. The results partially supported our hypotheses. Under ALAN, the crabs progressively changed their behavior along the course of the experiment. Although crabs spent more time out of the water at night, they did not increase hiding behavior and instead rested outside shelters, suggesting a low perceived predation risk above water. The increase in nocturnal resting behavior under ALAN was counterbalanced by increased daytime activity, suggesting a shift toward diurnal activity. MHWs induced emersion without affecting overall activity time consistent with our hypothesis. Under combined exposure, crabs increased nocturnal emersion and daytime activity, during which they preferred to stay underwater, hence showing complex responses to the stressors' interaction. Our results suggest that *P. marmoratus* shows high behavioral plasticity under different anthropogenic stressors, potentially enhancing its persistence in future Mediterranean rocky-shore environments.

Shedding Light on Marine Fish Societies in Dark Mediterranean Habitats: Sociality & Group Living in the Cardinalfish *Apogon imberbis*

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Uncovering how ecological and environmental conditions shape sociality in marine fishes remains a key frontier in behavioral ecology, particularly beyond the well-studied coral reef systems. The Mediterranean cardinalfish *Apogon imberbis*, a sciaphilic species inhabiting shaded rocky reefs, offers an opportunity to investigate how habitat structure and light environment influence group living. We examined whether group size and individual spatial behavior varied across four shaded habitat types—overhangs, caves, cracks and crevices—on a temperate rocky reef in Elba Island, Italy. Using SCUBA-based surveys, we quantified group size, habitat volume, and the degree of darkness for 30 social groups, and conducted focal observations of individual positioning relative to shelter. We found that group size was positively correlated with habitat volume, and habitat type constrained group size, with caves supporting the largest aggregations. Our findings also indicate that the proportion of darkness is related to habitat type (with greater proportions in caves versus cracks, crevices and overhangs) and that habitat type in turns influenced individuals' perceived predation risk and associated spatial behavior. More specifically, fish living in crevices, cracks and overhangs pay a cost due to need for increased vigilance compared to the ones living in caves: in habitats with less shading, individuals positioned themselves farther from shelter, while fish in caves remained closer to shelter compared to those in other habitat types. Additionally, larger individuals positioned themselves farther from shelter than smaller conspecifics. Together, these results demonstrate that variation in habitat structure and light environment strongly shapes social organization and spatial behavior in *A. imberbis*, highlighting the importance of incorporating diverse marine ecosystems into studies of social evolution.

Optical black holes enhance the detectability of riflebird displays: multi-level functionality of a visual signal

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Sexual selection is thought to drive the evolution of secondary sexual traits such as bright and complex colour patterns. While many bird-of-paradise species are famous for their extravagantly coloured plumage, some, like the riflebirds, are predominantly black, except for a few coloured patches. Signal theory suggests that achromatic contrast can be maximised by either increasing or decreasing brightness relative to the background. Here, through analyses of plumage brightness and spatial pattern information, we show that black body plumage in male riflebirds enhances detectability to prospecting females by creating an optical “black hole” that enhances both brightness and pattern contrast with the background. Due to limited colour transmission in dim and optically variable environments, selection may favour extremely dark, spatially regular body patterns to maximise background contrast. Together with previous work, our results suggest that female riflebirds are first attracted by male vocalisations before being visually guided to the display site by a conspicuous black pattern, after which they evaluate colour patches at close range during dynamic dance performances. Thus, black plumage may have a dual function as a conspicuous signal at long range and as a background for colour patches at close range. This kind of multifunctionality in signal components suggests that selection can have synergistic consequences for signal efficacy at different functional levels.

From Darwin’s Taste for the Beautiful to Today’s Beauty Bias: Research, Public Interest, and Conservation Efforts in European Butterflies

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From Charles Darwin’s early M and N Notebooks to mid-twentieth-century environmentalism, natural beauty was long viewed as a core driver for conservation. Today, however, contemporary conservation science increasingly treats beauty not as a value to protect, but as a “beauty bias” - a distortion shaping research priorities, public attention, and policy decisions. Our study addresses this phenomenon through an interdisciplinary approach integrating ecology, evolutionary biology, aesthetics, conservation science, and environmental governance. We conducted a multilingual online survey with over 21,000 respondents across 103 countries, gathering 170,000+ aesthetic evaluations of 492 European butterfly species. From these data, we developed a Species Beauty Index and linked it to a time-explicit socio-ecological network connecting species beauty, public attention, research effort, and major conservation frameworks - including the Bern Convention (1979), the EU Habitats Directive (1992), and the European IUCN Red Lists (2010, 2025). Our findings show that beauty bias consistently shapes public attention and scientific research, favoring widespread and visually attractive species. Early legal frameworks also disproportionately protected appealing and range-restricted taxa, generating long-lasting institutional effects that still influence modern funding and management priorities. By contrast, recent IUCN Red Lists increasingly identify less conspicuous species as threatened, revealing a growing mismatch between ecological risk assessment and historically embedded conservation priorities. Ultimately, aesthetics continues to structure human relations with biodiversity. Rather than rejecting it as a mere bias, we suggest returning to Darwin’s insights: re-understanding beauty as a powerful evolutionary and cultural force that conservation must critically confront and integrate into its scientific and institutional practices.



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**Symposium 3: “Phylogeography and Conservation
Genomics: understanding evolutionary history to
sustain species resilience”**

Chairs: Giulia Fabbri, Livia Tolve



Invited lecture

From Ancient Bottlenecks to Modern Populations: Genomic Consequences of Demographic Change

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Understanding how populations respond to demographic change is central to reconstructing evolutionary history and assessing contemporary extinction risk. In this talk, I will explore how population genomics can reveal the genomic consequences of demographic change across time, from ancient populations to modern conservation. I will first examine demographic bottlenecks associated with the dispersal of domestic animals from their centres of domestication, using ancient genomes to investigate how founder events, isolation, and subsequent population expansion shaped genetic diversity. I will then broaden this perspective to the extinct aurochs to examine patterns of diversification and demographic decline preceding extinction. Finally, I will discuss conservation genomics, showing how genomic data can be used to characterise contemporary population structure, genetic diversity, and demographic history, illustrated through case studies of the red panda and Asian elephants. Together, these examples demonstrate the genomic consequences of demographic change in completely different study systems and even time periods and how these perspectives on demographic change can help inform assessments of population resilience and extinction risk.

A treasure in the forest: the high diversity and structuring of the alpine longhorn beetle *Rosalia alpina* in Italy

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Rosalia alpina is a saproxylic longhorn beetle strictly protected under the European Habitats Directive and classified as Vulnerable by the International Union for Conservation of Nature. As a flagship and umbrella species, its conservation is crucial for the preservation of old-growth forest ecosystems, particularly in the current context of habitat fragmentation driven by land-use change, which represents a major threat to saproxylic beetle populations. We investigated the phylogeography and population structure of this species by sequencing a fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene and generating ddRAD data for 134 specimens from 37 sampling sites, covering a substantial portion of the species' Italian range. Our results highlighted the genetic uniqueness and variability of *R. alpina* populations in Italy, shaped by post-glacial recolonisation processes originating from southern Italian refugia in the Apennines and from Balkan source populations in the central-eastern Alps. Genomic analyses further revealed a clear separation between the above-mentioned evolutionary lineages, strongly supported by PCA (with PC1 explaining ~40% of the variance) and by high and significant pairwise F_{ST} values (0.60 and 0.69). Moreover, within the Apennines, two main genetic clusters were detected, separating central-northern and southern populations. This pattern likely reflects both historical and recent habitat fragmentation events that allowed limited gene flow across central Italy. Further sampling in the central Apennines and central Alps will be essential to better characterise gene flow dynamics and support conservation strategies aimed at preserving the genetic uniqueness of Italian populations in the face of ongoing old-growth forest loss and fragmentation.

Genomic phylogeography reveals a hotspot of *Luciola* fireflies on Western Mediterranean islands

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Islands are priority areas for biodiversity research and conservation, as they often harbour distinct evolutionary lineages, yet they are also particularly threatened by global change. In the context of the accelerating biodiversity crisis, many insular species may disappear before they are even discovered. Fireflies, despite their ecological and cultural significance, remain poorly investigated, especially in temperate regions, where marked declines have been reported over recent decades. We investigated the geographic structure of genetic variation in *Luciola* fireflies from the Tuscan Archipelago and the adjacent Italian peninsula using an integrative phylogeographic framework based on mitochondrial and genome-wide nuclear data. We identified two distinct, deeply divergent, and geographically structured lineages restricted to Elba Island, with no evidence of gene flow with populations from the Italian peninsula. Within Elba, the two lineages showed fine-scale population structure, with a scattered east–west spatial pattern and a narrow admixture zone mainly restricted to the central part of the island. This pattern suggests limited gene flow and ongoing reinforcement of reproductive isolation. Interestingly, the two Elba lineages were closely related to each other, but distantly related to both the peninsular and Sardinian-Corsican lineages, supporting a scenario of ancient colonization followed by in situ diversification. Overall, our study identifies the Tuscan Archipelago as a micro-hotspot of biodiversity and evolutionary processes promoting biological diversification, highlighting its conservation priority and irreplaceable evolutionary heritage.

Demographic responses of wild bees to the Anthropocene: a global comparative genomic approach

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Understanding how populations have responded demographically to anthropogenic pressures is a central challenge in conservation biology, as such responses determine both current vulnerability and future persistence. Over the last two centuries, industrialization, land-use change, agricultural intensification, and climate change have reshaped natural environments worldwide, yet broad comparative assessments of their demographic consequences across species and regions remain scarce. Population genomics now provides the resolution needed to reconstruct recent demographic histories and to place contemporary biodiversity patterns within a historical framework. Here, we present a large-scale comparative study aimed at quantifying recent demographic trajectories of wild bee populations at a global scale. We compile population genomic datasets from more than 20 bee species representing multiple families and continents, and use demographic inference to estimate temporal changes in effective population size and patterns of genetic diversity across regions. The primary aim of this study is to identify shared and divergent demographic responses across countries and species and to assess the influence of Anthropocene environmental change on population trajectories. This study goes beyond single-species or local case studies by adopting a comparative, cross-taxonomic framework to evaluate how demographic responses to recent global change vary. By synthesizing population genomic studies from wild bees worldwide, this study outlines a scalable approach for integrating population genomics into conservation-relevant assessments of population history. Such an approach is essential for interpreting present-day genetic variation, informing biodiversity monitoring, and supporting long-term conservation planning under ongoing environmental change.

Second-chance speciation in the Clouded Apollo butterfly: genomic and chemical evidence of reproductive isolation

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Understanding how reproductive barriers emerge among recently diverged lineages at secondary contact is a challenge in phylogeography. The Clouded Apollo (*Parnassius mnemosyne*) is a European butterfly protected under the Habitats Directive, yet its taxonomic stability is challenged by evidence for genetic divergence in two spatially clustered lineages. We employed an integrative approach, combining genome-wide SNP markers, Cuticular Hydrocarbon (CHC) analysis and wing-scale geometric morphometrics with ecological and demographic modelling, to reconstruct the evolutionary history of this taxon and evaluate lineage boundaries. We confirmed the existence of two lineages, *P. mnemosyne* and *P. turatii*, which diverged ~0.8 million years ago. Through historical demographic inference we identified two episodes of secondary contact: the first, during the last glaciation, led to asymmetric gene flow and admixed populations in the Pyrenees. The second occurs at the present Eastern Alps contact zone where we detected no gene flow and no recent introgression. This indicates that speciation is now complete, despite morphological and ecological similarity between the groups. We provide evidence for chemical reinforcement: CHC profiles exhibit significant mutual character displacement specifically at the contact zone, 96-100% classification accuracy, decaying with geographic distance. This suggests that chemical signalling acts as premating barrier refined during secondary contact. Conversely, wing-scale morphology converges in the contact zone, rendering it unreliable for species diagnosis in sympatry. These findings portray a system of "second-chance speciation" in which lineages that once exchanged genes subsequently evolved reproductive isolation in the Alps. This

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understanding evolutionary history to sustain species resilience”

discovery has implications for conservation genomics, suggesting that current IUCN assessments and European legislation may need to be updated to protect these evolutionary identities and their unique genomic heritage

Small Populations, Lasting Signatures: Demography and Genetic Load in the Insular Endemic Butterfly *Hipparchia sbordonii*

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Widespread insect declines raise concerns that genomic erosion, the loss of genetic diversity with increased inbreeding and deleterious variation, may also affect insects, which are usually assumed to be buffered by large population sizes. We investigated this issue in the Ponza grayling, *Hipparchia sbordonii* (*H. sbordonii*), an endangered butterfly endemic to the Pontine Islands, and compared it with the widespread mainland sister species *Hipparchia semele* (*H. semele*). We analysed whole-genome resequencing data from historical and contemporary *H. sbordonii*, contemporary *H. semele*, and two outgroup to reconstruct demographic history, estimate genetic diversity, quantify runs of homozygosity (ROH), and assess genetic load through functional annotation and ancestral-state polarization. Demographic analyses revealed a sustained contraction in *H. sbordonii*, including a sharp decline over the last 30 generations, whereas *H. semele* maintained a much larger and stable population size. Consistently, *H. sbordonii* showed reduced genetic diversity, elevated ROH abundance and length, and higher realized genetic load, indicating more deleterious variants in homozygous state. In contrast, *H. semele* showed higher masked load, consistent with deleterious variants being maintained mainly in heterozygous state in a larger population. Demography-corrected relative derived allele frequency analyses (R'_{xy}) suggested only limited depletion of high-effect derived mutations in *H. sbordonii*, providing weak evidence for purging, while moderate- and low-effect mutations were enriched, consistent with the accumulation of weakly deleterious variation under drift and reduced purifying selection. Overall, our results

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understanding evolutionary history to sustain species resilience”

show that small and isolated insect populations can experience genomic erosion comparable to that documented in declining vertebrates.

Gene presence/absence variation reveals the evolutionary history of the Australian blue mussel *Mytilus planulatus*

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Blue mussels of the globally distributed *Mytilus edulis* species complex are key ecological foundation species and major aquaculture resources. In Australia, they were historically classified as *M. galloprovincialis*, a species native to the Mediterranean and northeast Atlantic, that has invaded large parts of the globe through human-mediated transport. However, in Australia, SNP-based studies show that native populations should be considered as a distinct species, *Mytilus planulatus*, which nevertheless shows evidence of introgression with *M. galloprovincialis*. However, the lack of genetic resources for southern hemisphere populations of *Mytilus* has prevented disentangling the complex evolutionary history of the species complex in this geographical range. We generated the first reference genome for *M. planulatus* and resequencing data from three Australian populations: a putatively pure Tasmanian population, and two previously described hybrid populations putatively introgressed with genetically distinct *M. galloprovincialis*, from the Mediterranean Sea and Atlantic Ocean. Given the extensive structural variation characteristic of *Mytilus* genomes, we investigated population structure using both SNPs and gene presence/absence variation (PAV), integrating our data with global resequencing datasets. Our results support previous studies, but suggest that Southern Hemisphere colonisation may have occurred in multiple waves, followed by recent human-driven migrations that overall explain the complex admixture patterns observed. We further show that genes displaying PAV are enriched for immune-related functions, suggesting that gene gain and loss may have contributed to rapid adaptation to local pathogen pressures during range expansion. Together, these results highlight the importance of dispensable immune repertoires in *Mytilus* evolution and have implications for the management of the native populations of Southern Hemisphere blue mussels.

Chloroplast capture in forest trees: evidence from Mediterranean oaks and alders and evolutionary implications

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Chloroplast capture, i.e. the introgression or fixation of a chloroplast genome from one species into another through hybridization and repeated backcrossing, is increasingly recognized as a pervasive evolutionary process in plants. This phenomenon is particularly common in long-lived, wind-pollinated trees, where incomplete reproductive barriers and extensive gene flow facilitate interspecific introgression across geographic and temporal scales, but its evolutionary and phylogenetic implications remain insufficiently explored. Here, we present comparative evidence of chloroplast capture in two groups of Mediterranean trees investigated within the framework of the National Biodiversity Future Center (NBFC PNRR), based on complete chloroplast genomes reconstructed from whole-genome resequencing data. The first dataset includes 70 individuals of the Italian alder (*Alnus cordata*) and 29 individuals of the common alder (*A. glutinosa*), sampled across the entire Italian distribution range of the former species. The second dataset comprises 38 individuals representing 12 Mediterranean cork oak species (*Quercus* subg. *Cerris* sect. *Cerris*), covering the entire distribution area. Phylogenomic analyses revealed signatures of chloroplast capture in both study systems, resulting in geographically structured sharing of plastid haplotypes among different species, as well as conflicts between plastid- and nuclear-based phylogenies. Our results, together with previous evidence from literature, show that chloroplast capture is a widespread and recurrent phenomenon among wind-pollinated forest trees. We will discuss how chloroplast capture may affect species delimitation, phylogenetic inference and phylogeographic reconstruction. Finally, we will highlight future research directions, including the integration of organellar and nuclear genomic data, and the study of the role of hybridization and introgression in shaping adaptive responses of forest trees under ongoing climate change.

A target-capture approach for genomic insights into the postglacial expansion of the Italian endemic amphibian *Bombina pachypus*

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Geographic range expansion affects population dynamics and shapes biodiversity distribution. Dispersion and colonization of new habitats may lead to genetic diversification as populations adapt to environmental changes. In this study, we present a target-capture approach developed to investigate genomic variation associated with postglacial expansion in the Italian Apennine yellow-bellied toad (*Bombina pachypus*). Like many amphibians, *B. pachypus* possesses a large and complex genome (~10 Gb), making whole-genome resequencing costly for population-scale studies. To overcome these limitations, we developed a custom target-capture panel targeting approximately 24 Mb of the genome, including exons from genes with different expression levels, regulatory sequences identified through open chromatin sequencing (ATAC-seq), and putatively neutral intergenic regions, enabling the simultaneous characterization of coding and non-coding variation across genomic regions experiencing different selective pressures. Probe design was optimized to maximize capture efficiency while maintaining a cost-effective sequencing strategy suitable for large-scale population genomic analyses. The panel was experimentally validated on 24 individuals across the genus *Bombina*, including 16 *B. pachypus*, 4 *B. variegata*, and 4 *B. orientalis*. Capture performance demonstrated high efficiency in the target species and successful enrichment in congeners, highlighting the robustness of the approach. This method provides an effective framework for investigating genetic diversity, genetic load, adaptation, and evolutionary history in non-model amphibians with large and complex genomes.

Whole-genome analyses uncover population structure and evolutionary processes in leatherback turtles across ocean basins

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The leatherback turtle (*Dermochelys coriacea*) is classified as Vulnerable by the International Union for Conservation of Nature (IUCN), with several populations undergoing rapid declines. Previous studies based on mitochondrial DNA (mtDNA) revealed extremely low variation, limiting the resolution of population structure and connectivity analyses. Advances in high-throughput sequencing and the availability of a reference genome now enable genome-wide analyses at unprecedented resolution. Using whole-genome resequencing, we aim to determine the genomic structure of species across five Regional Management Units (RMUs): Northwest Atlantic, Southwest Atlantic, Southeast Atlantic, East Pacific, and West Pacific.

TALKS - Symposium 3: “Phylogeography and Conservation Genomics:
understanding evolutionary history to sustain species resilience”

Genome-wide single-nucleotide polymorphisms (SNPs) revealed strong population structure among RMUs through Principal Component Analysis (PCA), indicating long-term evolutionary divergence and restricted gene flow across ocean basins. Genomic assignment of 23 individuals sampled in Spanish waters showed most belonged to the Northwest Atlantic RMU, emphasizing the importance of integrating nesting and foraging areas into conservation frameworks. Genome-wide differentiation analyses identified regions with elevated F_{ST} , suggesting putative adaptive divergence among RMUs. We characterized runs of homozygosity (ROHs) and genetic load to assess genomic vulnerability. ROH segments were mostly short, consistent with historical inbreeding. Populations with higher ROH accumulation also exhibited increased genetic load, suggesting reduced efficacy of purifying selection and potentially lower adaptive potential. Finally, demographic reconstructions revealed distinct evolutionary trajectories among populations. Our results demonstrate how whole-genome data can uncover evolutionary processes shaping marine turtle populations and provide key insights for conservation strategies aimed at preserving genomic diversity and adaptive potential under ongoing environmental change.

Reconstructing complex demographic histories in reef sharks using a metapopulation ABC framework and sex-specific genomic contrasts

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Inferring demographic history and the forces shaping genomic variation is a central challenge in evolutionary biology with direct relevance for conservation. In widely distributed marine species environmental heterogeneity and life-history traits (.e.g dispersal, philopatry) interact across to generate complex metapopulation structures that often violate assumptions of standard demographic models. The grey reef shark (*Carcharhinus amblyrhynchos*), an endangered predator that has undergone severe population declines, exemplifies these dynamics. Here, we generate a new reference genome for *C. amblyrhynchos* and analyse whole-genome data from hundreds of individuals across its range. We combine these data with our novel ABC framework which extends standard demographic models (IM, SC, AM, SI) to incorporate metapopulation structure. Together with chromosome-specific analyses, this approach allows us to test alternative scenarios and quantify the genomic consequences of sex-biased processes. We find strong support for two ancient Indo-Pacific metapopulations that evolved independently for ~1 million years, with recent and limited secondary contact. Asymmetries in the SFS indicate distinct centres of expansion, while selection scans suggest adaptive differentiation within metapopulations. Comparisons between autosomes and the X chromosome reveal extreme reductions in X-linked diversity and elevated differentiation, with estimates of the effective sex ratio (Q) between 0.1 and 0.5, well below the neutral expectation of 0.75, and differing between metapopulations. Their long-term independence, strong differentiation, and distinct sex-biased signatures support recognition as separate Evolutionarily Significant Units. More broadly, our results show how metapopulation-aware demographic models resolve complex histories, while chromosome-specific analyses reveal the effects of sex-biased dispersal on genomic diversity, with implications for defining conservation units.

Population Puzzles: Understanding the Genetic Structure and Health of Recently Diverged Galapagos Giant Tortoise Lineages

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The Galapagos giant tortoises (*Chelonoides* spp.) are a notable example of rapid evolution and diversification in islands. The southern region of Isabela Island is home to population fragments of the most recently diverged lineages, showing a morphological gradient from West to East. Two species have been named here – *C. vicina* associated with the Eastern volcano Cerro Azul, and *C. guntheri* associated with the Western volcano Sierra Negra. The geographic extent and taxonomic affiliation of each population fragment in the present day is not well understood and as a result, neither is their genetic health. This understanding is critical in order to plan future conservation actions, especially in the case of the Sierra Negra population (possibly the only remaining of *C. guntheri*), which has been greatly diminished due to historical hunting and ongoing poaching. We used whole genomes from individuals sampled across Southern Isabela to assess population structure using clustering and phylogenetic approaches. After grouping sampling locations into genetic clusters, we assessed population genetic health through heterozygosity, nucleotide diversity, and identification of runs of homozygosity (ROH). Additionally, we also assessed levels of migration between the clusters. We find that, in Southern Isabela, there are eight distinct genetic clusters, with only one belonging to *C. guntheri* and not much movement between clusters. However, we also note that the nucleotide diversity, heterozygosity, as well as the number and total length of ROH are comparable across clusters, with no cluster showing clear signs of vulnerability. We therefore conclude that the focus for current conservation action should be on increasing population size for the one *C. guntheri* population with no immediate need for translocations among the *C. vicina* fragments.

Mitogenomic phylogeography of the gray wolf (*Canis lupus*): ancient and contemporary data clarify the phyletic relationships across wolf populations and canine clades

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The increasing availability of genomic and paleogenomic data and tools has greatly improved our ability to reconstruct the evolutionary history of species, yet the phylogeography of the gray wolf (*Canis lupus*) and the origin of dog lineages remain only partially resolved. We assembled one of the most comprehensive mitogenomic datasets to date, comprising 950 wolf and dog mitochondrial genomes spanning the entire Holarctic region and a temporal range from ~67 kya to the present, including newly generated contemporary European and ancient Italian samples. Phylogenetic (ML and Bayesian) and tip-dated coalescent analyses were used to reconstruct evolutionary relationships, divergence times, and spatiotemporal dynamics of wolf and dog lineages. Our results confirm that most contemporary wolf mitochondrial diversity is structured into two major haplogroups (W1 and W2) and reveal a third paraphyletic assemblage (W3), composed mainly of ancient European and Beringian lineages. Temporal analyses indicate a marked turnover, with W3 dominating during

TALKS - Symposium 3: “Phylogeography and Conservation Genomics:
understanding evolutionary history to sustain species resilience”

the Pleistocene and being largely replaced by W1 and W2 around 20 kya. Contrary to previous hypotheses of a single Beringian expansion during the late Pleistocene–early Holocene, our findings support a scenario involving multiple refugia in Europe and Beringia, driving repeated dispersals and lineage replacements. Dog haplogroups show a complex origin, involving multiple maternal contributions from a structured and partially extinct wolf population. Dog haplogroup A falls outside both W1 and W2, suggesting a derivation from a more ancient wolf lineage. Overall, our results provide a refined reconstruction of the wolf matrilineal history, highlighting extensive loss of ancient diversity and supporting a complex, reticulate scenario for dog domestication involving a main ancestral source plus multiple additional matrilineal contributions.

From immunity to diet: potentially adaptive loci in the Apennine brown bear

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The Apennine brown bear (*Ursus arctos marsicanus*) is among European's most critically endangered and genetically isolated large carnivore populations, raising concerns about the maintenance of adaptive genetic variation. To place this population in a broader evolutionary context, we examined immunity- and diet-related candidate genes in 89 brown bears spanning the species' range, from Scandinavia and Central Europe to North America and Russia, with four polar bears as outgroup. Candidate genes were identified through ortholog searches, validated via BLAST, and variants were called within coding regions using a high-quality filtering pipeline. Population genetic statistics (π , Tajima's D, FST, pN/pS) were computed to flag genes showing unusual variation or divergence. Among diet-related genes, pancreatic alpha-amylase (AMY) showed uniform copy number (~6 diploid copies) across all Apennine bears, contrasting with broader variation in other populations. The sweet taste receptor TAS1R2 carried thirteen nonsynonymous mutations across functional domains, several fixed or at high frequency in Apennine bears, suggesting functional divergence in taste perception. Bitter taste receptor genes (TAS2R39, TAS2R41) also showed detectable variability, supporting a role for sensory genes in ecological adaptation. Immune-related genes displayed heterogeneous patterns: interleukins, KLR-family, and Toll-like receptors showed the highest diversity, while chemokine genes were highly conserved. Functional differences emerged between membrane-bound and endosomal TLRs. Overall, despite extremely reduced genome-wide diversity, the Apennine bear retains functionally relevant variation at multiple candidate loci, possibly reflecting balancing selection, paralog divergence, or local adaptation. These findings broaden our understanding of natural selection in small isolated populations, with important implications for the design of conservation strategies that account for adaptive variation.

Genomic vulnerability at the brink of extinction: genetic load in the giant deer *Megaloceros giganteus*

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TALKS - Symposium 3: “Phylogeography and Conservation Genomics:
understanding evolutionary history to sustain species resilience”

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The giant deer (*Megaloceros giganteus*) was one of the largest and most widespread cervids of the Late Pleistocene, ranging from western Europe to eastern Asia. Despite its iconic status, little is known about its genomic history prior to extinction (ca. 8 kya), leaving open the question of whether it was already genomically compromised before its disappearance. We generated the first nuclear palaeogenomes from nine individuals from Germany (ca. 40 kya) and Ireland (ca. 11 kya), mapped to a new chromosome-level reference genome of the fallow deer (*Dama dama*). Demographic reconstructions revealed a long-term history of low effective population size, with a pronounced decline beginning around 60 kya. To understand if this decline actually promoted the accumulation of deleterious mutations, we compared genetic load between the German and Irish samples. Irish individuals carry a higher burden of high-impact derived homozygous genotypes than German individuals. However, normalizing against neutral sites reveals a signal of partial purging of strongly deleterious alleles in the Irish population. This pattern is consistent with continued purifying selection acting during demographic contraction and is analogous to what has been documented in other Late Quaternary megafauna, including the woolly mammoth. Nevertheless, when compared to ten modern cervid species with varying demographic histories, the Irish *M. giganteus* retains one of the highest genetic loads among all cervid species considered, closely matching values observed in *Axis porcinus* and *Cervus hanglu yarkandensis*, two taxa of conservation concern. Thus, although some signal of purging is detectable within *M. giganteus*, its overall burden

TALKS - Symposium 3: “Phylogeography and Conservation Genomics:
understanding evolutionary history to sustain species resilience”

of deleterious alleles remained elevated compared to most extant cervids, suggesting that the species entered the terminal Pleistocene in a genomically vulnerable state.



I think



Symposium 4: “Human evolution”

Chairs: Silvia Ghirotto, Stefania Vai



Invited lecture

Unlocking the Past: Twenty Years of Paleogenomics

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Over the past two decades, paleogenomics has transformed the study of human evolution, evolving from a technically challenging discipline into one of the most powerful approaches for investigating our evolutionary past. Ancient genomes have provided unprecedented insights into population history, migration, adaptation, kinship, and the long-term interactions between humans and pathogens, fundamentally reshaping our understanding of the human past. The major milestones that have defined the field will be explored through selected case studies, illustrating how paleogenomic research has addressed long-standing questions in biological anthropology and human evolution. Looking ahead, the expanding range of substrates suitable for ancient DNA analysis—including sediments, dental calculus, coprolites, museum specimens, historical collections, and other unconventional biological archives—is opening exciting new research opportunities. By extending genomic investigations beyond skeletal remains, these emerging resources are bridging paleogenomics with the rapidly developing field of Museomics, where museum collections are becoming invaluable genomic archives for reconstructing the evolutionary history of our species and the biological interactions that have shaped it through time.

Family ties and population structure in a Copper Age collective burial: the case of Corna Nibbia

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Understanding the evolution of the social structure of prehistoric communities is a crucial aspect of our species' history. Archaeologists investigate this by analyzing entire necropolises, where tomb location, grave goods, and body arrangement provide important clues about community social structures. Today, archaeological evidence can be integrated with the analysis of ancient genomes to explore the social and familial dynamics of past communities, as well as to infer their population structure and migration patterns. In this study, we analyzed the Copper Age site of Corna Nibbia (Bione, Italy). This necropolis is a collective burial site characterized by scattered and disarticulated human remains, likely resulting from complex funerary practices involving secondary burial and post-depositional manipulation. Anthropological analyses estimate a minimum of 29 individuals, of which 22 skeletal remains were selected for DNA analysis. Among these, 9 petrous bones yielded sufficient endogenous DNA for genomic sequencing, resulting in whole-genome data for 7 individuals with an average coverage of 1x. The remaining 2 samples are currently undergoing whole-genome enrichment sequencing. Analysis of uniparental markers reveals high mitochondrial diversity, while all identified male individuals share the same Y-haplogroup, suggesting a patrilocal organization with female exogamy. Kinship analysis identified close biological relationships, including a father–daughter pair, and a probable grandparent–grandchild pair. We compared the Corna Nibbia individuals with over 300 samples spanning from the Mesolithic to the Middle Ages. Our results show that they fall within the genetic variation of coeval European populations, with predominant Anatolian farmer ancestry and an additional contribution from Western Hunter-Gatherers. These findings highlight the importance

of integrating paleogenomics with archaeology to better understand the social dynamics of prehistoric communities.

Crossing the Sea: a paleogenomic perspective on the main Italian Mediterranean islands

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The Mediterranean has always played a key role in the peopling processes and cultural shifts that have shaped Europe over the centuries and millennia. Among the areas of particular interest for the study of these dynamics, the large Mediterranean islands offer a privileged context due to their strategic position and abundance of archaeological evidence. However, despite their importance, the contribution of these islands to the genetic history of the Mediterranean requires further investigation: the extension of the sampling and the time horizon, as well as the increase in the information potential of the genetic data produced, would help to overcome the limitations of the studies already available in the literature. This is the context for the PRIN2020 “Crossing the Sea” project, which is mainly aimed at reconstructing the genetic history of the main Italian Mediterranean islands from the Upper Palaeolithic onwards, through the analysis of ancient and modern genomic data using a multidisciplinary approach involving archaeologists, anthropologists, palaeoanthropologists and population geneticists. The paleogenomic analyses involved over 260 ancient individuals from Sicily and Sardinia. From this set, more than 60 individuals have been selected so far, based on molecular preservation, yielding whole-genome data with a mean coverage between 0.1x and 5.3x. Starting from Sicily, different computational approaches, such as genotype imputation and genotype likelihoods, were applied according to genome coverage. Subsequent analyses including PCA (Principal Component Analyses), admixture, and IBD (Identity By Descent) were performed. The results made it possible to outline the genetic history of Sicily and to compare it with the Italian peninsula and Europe. Future

analyses will involve the data obtained for the Sardinian samples, and, in addition, in-depth studies will be carried out on specific sites to investigate local dynamics in a greater detail.

***Marcus Venerius Secundio*: a molecular portrait of a uniquely preserved individual from Pompeii**

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Pompeii, a Roman colony from the late Republican period, developed into a significant urban centre. Within this context lies the case of *Marcus Venerius Secundio*, an exceptionally well-preserved individual recovered from the Porta Sarno necropolis, who died shortly before the eruption of 79 CE. We sequenced his genome, obtaining a mean coverage of 5×, and analyzed it through a genotype-likelihood-based approach. In parallel, we performed a metagenomic analysis aimed at identifying potential ancient pathogens. We compared the genetic variation of *Marcus Venerius Secundio* with that of hundreds of ancient individuals spanning from the Paleolithic to the Medieval era across Europe, Anatolia, the Near East, the Caucasus, the Levant, North Africa and sub-Saharan Africa, highlighting affinities with Near Eastern populations. Consistently, Admixture analyses reveal a predominant ancestral component related to eastern–Caucasian groups, accompanied by a smaller Anatolian contribution and an Eastern Hunter-Gatherer fraction. This signal is further corroborated by mobility pattern analyses and by the inference of pigmentation phenotypes, which suggest a non-local origin, most plausibly in the southern Caucasus region, potentially corresponding to present-day Georgia. Uniparental markers provide additional support for a non-local origin: the mtDNA belongs to haplogroup U3b3, while the Y chromosome is J-Z7706, that are today present at the highest frequencies in the Near East and the Caucasus. Overall, the genomic,

phenotypic and uniparental evidence indicates that *Marcus Venerius Secundio* had an extra-Italian eastern origin. Considered alongside the distinctive funerary context of his tomb, characterized by the exceptional use of inhumation within a Pompeian funerary landscape in which cremation was the prevailing practice, these results further emphasize the significance of this individual within the broader archaeological framework of Pompeii.

Genomic Threads of Indigenous Americans: Reconstructing Population Dynamics and Environmental Adaptation in Panama, Peru, and Ecuador

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The intricate history of Indigenous Americans is far from being fully reconstructed due to the limited number of high-coverage genomes available. Here, we analyzed 70 high-quality, unrelated genomes representing diverse Indigenous individuals (including a few admixed individuals) from various geographical locations in Panama, Peru, and Ecuador to reconstruct their genetic structure, population dynamics, and demographic history. We also performed scans for positive selection and identified signatures of high-altitude adaptation in the Kichwa, implicating regulatory, metabolic, and cellular stress pathways rather than canonical hypoxia genes alone. In contrast,

a replicated signal at the IPCEF1–OPRM1 locus across Ashaninka and Panamanian populations indicates a shared adaptive response among lowland groups, consistent with environmental pressures associated with tropical ecosystems.

Reassessing Quinzano 1 (Verona): a morphometric investigation of Neanderthal affinities in a human occipital fragment.

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The Italian Peninsula is a pivotal region for investigating the evolution and dispersal of archaic human populations during the Pleistocene. Among the available fossil record, a human occipital fragment recovered in 1938 at Quinzano (Verona) has remained poorly investigated. The stratigraphic context of the specimen remains uncertain due to the complexity of the alluvial sequence and its recovery during quarrying activities; however, associated taphonomic, faunal, and archaeological evidence supports an attribution to the Middle–Late Pleistocene transition. Quinzano 1 consists of a partial occipital squama, preserving the lambdoid suture from the near-asterionic region to the lambda; however, the anterior portion is fractured at the level of the superior nuchal line. The specimen was analyzed using micro-CT scanning (voxel size: 67.8 µm). After assessing the preservation of the sutural margins and identifying key osteometric landmarks, two primary analyses were performed: a detailed morphological examination of the suprainiac fossa – a recognized Neanderthal autapomorphy – and a geometric morphometric (GM) study of the occipital squama using a surface registration technique. Surface registration allows to study the full morphology of a fragmented specimen even when few osteometric points are available. The 3D comparative dataset included Neanderthals, Middle Pleistocene European hominins, and a diverse *Homo sapiens* sample. Results confirm the presence of a suprainiac fossa, and GM analysis indicates strong morphological affinities with both Neanderthals and Middle Pleistocene fossils. These findings suggest that Quinzano 1 most likely represents a Neanderthal individual. While a precise chronological framework remains elusive, future research should prioritize direct dating and a more precise reconstruction of the site’s archaeological context.

Taung's Hidden Smile: The Morphology of Permanent Teeth Revealed a Century Later

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The Taung fossil has played a central role in the historical development of paleoanthropology, redirecting the interest of earlier scholars to the African continent as the cradle of humankind. This specimen is the Holotype of the species *Australopithecus africanus* and was first published by Dart in 1925. In the 100 years following Dart's original publication, numerous works have focused on Taung's dentition, associating the Taung specimen with fossils from the sites of Sterkfontein and Makapansgat, and distinguishing it from those from Swartkrans, Kromdraai, and Drimolen. While the pattern of the skull's developing permanent dentition has been investigated radiologically, the morphology of these teeth has not yet been examined in detail using advanced radiographic methods. This work contributes to our knowledge of the permanent dentition of the Taung specimen through the employment of micro computed tomography (microCT) scans of the maxilla and mandible to describe and provide metrical information on the crowns of its permanent teeth. The permanent dentition was segmented from microCT scans although the high density and heavy mineralization of the bone surrounding the developing teeth, along with the occurrence of calcite encrustations on some of the crowns hindered resolution of some fine-scale morphological details. We present preliminary descriptions of the outer enamel surfaces of the developing permanent teeth. We also recorded standard MD and/or BL crown measurements from our segmented 3D models of the unerupted dentition using Avizo v. 7.1 software. Linear measurements were compared to samples of *A. africanus* (specimens from Sterkfontein and Makapansgat) and *Paranthropus robustus* (specimens from Kromdraai, Swartkrans, Drimolen, Cooper's. Gondolin, and Sterkfontein Member 5). Overall, the MD and BL diameters of the Taung infant's developing permanent crowns show similarities with the *A. africanus* sample and notable differences from the *P. robustus* dental sample.

The evolutionary roots of war and peace

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This article leverages the comparative method to examine the evolutionary roots of intergroup lethal conflict and peaceful cooperation through a cross-species comparison of chimpanzees, bonobos and human hunter-gatherers. It asks whether patterns of intergroup relations were inherited from the last common ancestor of *Pan* and *Homo* (LCA_{P-H}) (homologies, evolved independently within particular lineages (autapomorphies), or arose through convergent evolution (analogous traits). The paper first clarifies the methodological value and limitations of comparing extant species and of using palaeoanthropological and ethnographic evidence to reconstruct the evolution of intergroup relations in *Pan* and *Homo*. It then reviews intergroup relations in the two *Pan* species. Despite significant variations across sites, chimpanzees display border patrols and coalitionary killings, including lethal aggression against members of neighboring communities. Bonobos, by contrast, show greater variability and tolerance in intergroup encounters, although specific patterns differ across study sites: while they do engage in aggression (with two suspected cases of intragroup lethal aggression), they do not exhibit intergroup coalitionary killings and can engage in cooperation (e.g. food-sharing and coalitions formation) between individuals across groups. The article next turns to humans, focusing on ethnographic evidence from nomadic hunter-gatherers. This record indicates both intergroup lethal conflict and extensive group-level peaceful cooperation. Finally, drawing on cross-species comparisons and the fossil record of Miocene apes and hominins, the paper examines what can and cannot presently be inferred about the LCA_{P-H}. It stresses the uncertainty of direct reconstruction, while highlighting likely ancestral male-male aggression and the derived character of large-scale, group-level peaceful cooperation in hominins.



I think



Symposium 5: “The evolution of ecological diversification: mechanisms, interactions, and outcomes”

Chairs: Elisabetta Bianchi, Andrea Chiocchio, Ivan Scotti



Invited lecture

Diversity in plant hydraulic traits across scales: evolutionary constraints and adaptive potential

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Processes of uptake, transport and regulation of water use vary tremendously in plants around the three classical strategies of dehydration resistance, avoidance and escape. Our understanding of the macro and micro-evolutionary processes controlling plant water relations and plant hydraulic traits is still preliminary, however advancing our knowledge in this field is necessary in the context of a rapidly changing climate, especially with already occurring shifts in rainfall intensity and frequency, drought and heat waves severity and evidence of drought-induced plant mortality across the globe. I will review the progress in this field, focusing on macro-evolutionary changes in some of the most important traits controlling plant water use, the limits of plasticity in this traits, the ecological significance of inter-population and intra-population trait diversity and the evidence for population genetic differentiation, with a special focus on trees, both at the global and the European scale.

The Budbreak Variations: Differential genetic and environmental control of bud break between populations and years in European beech

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Deciduous trees track the local climate through phenological events that result from the interplay between the genotype and the environmental cues. The time of budbreak (TBB) is pushed earlier or pulled later by temperature cues toward a longer vegetative season and the avoidance of spring frost. Thus, the trait has adaptive value

and is highly heritable. We performed in situ genome-wide association study (GWAS) to investigate the genetic bases of phenotypic variation in TBB in two range-edge glacial refugia of European beech (*Fagus sylvatica* L.): La Massane Reserve (Pyrenees, South-West France) and Mont Ventoux (Provence, South-East France). TBB was recorded in two consecutive years on 150 (La Massane, 2022 and 2023) and 162 trees (Mont Ventoux, 2009 and 2010). Besides TBB, we described and analysed nine additional traits representing facets of spring phenology. About 2 million SNPs were called on two reference genome assemblies from different phylogeographic lineages. We found 17 (La Massane) and 58 (Mont Ventoux) QTLs associated with spring phenology; they were exclusive to each population and year-specific. Mixed models support a substantial contribution of genotype-by-environment interactions in driving the trait under different climatic conditions. The QTLs explained between 0.2 and 59% of phenotypic variance, suggesting a polygenic architecture with multiple small-effect loci and a few loci with large effects. Bootstrapping led to non-overlapping results (R^2 : 0.2 - 17.6). Few SNP hits were shared between assemblies, possibly due to replicated regions. Our findings highlight how the genomic architecture of spring phenology is shaped mostly by local processes: local environmental pressures select combinations of adaptive alleles that depend on the phylogeographic background, whilst the season's climate modulates the genetic effects through extensive genotype-by-environment interactions. This results in population-specific mechanisms of genetic control of TBB.

The INTERACT project: Understanding evolution of common bean plant-plant interactions.

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Agroecosystems have been shaped by crop domestication and modern breeding, processes that simplified biotic interactions and reduced genetic diversity in cultivated systems. Climate change highlights the need to better understand plant–plant interactions to improve agricultural sustainability and resilience. Despite their ecological and agricultural relevance, intraspecific interactions remain poorly explored. This study investigates interactions between *Phaseolus vulgaris* plants using transcriptomic, metabolomic, and rhizosphere metagenomic analyses. The evolutionary history of *P. vulgaris*, characterized by two independent domestication events in Mesoamerica and in the Andes, provides a replicated framework to explore how domestication reshaped interaction-dependent responses. Eighteen genotypes, six in each of the wild, landrace, and cultivar groups were selected from previous genomic studies. Controlled experiments evaluated 90 genotype combinations in a randomized block design. Root, leaf, and soil samples were collected for multi-omics analyses. Transcriptomic analyses of the Mesoamerican gene pool revealed a domestication signature, with roots showing stronger transcriptional divergence than leaves. While a conserved set of domestication-associated genes was maintained across domestication stages, the limited overlap of differentially expressed genes among stages indicates that domestication and breeding affected distinct regulatory pathways. Transcriptional responses strongly depended on neighbor identity, with roots and leaves showing distinct responses to cultivar neighbors across focal

genotypes, suggesting that cultivars are associated with stronger interaction-dependent transcriptional responses. Roots showed enhanced transcriptional reprogramming, highlighting the role of belowground responses in eco-evolutionary dynamics. Metagenomics also revealed shifts in rhizosphere microbial composition associated with domestication level and neighbor identity.

Demographic inference from genomic and ecological data uncovers Human linked decline in Squamata reptiles

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The global vertebrate's distribution and diversity results from complex ecological, life-history, evolutionary and climatic interactions. The Squamata order, with over 10,000 species adapted to diverse habitats and showing extreme life-history variation, such as adult body size, represents an ideal model to explore how broad-scale biogeographic processes and intrinsic biological traits affect demographic history and genomic diversity. To investigate the factors that mainly influenced the demography of Squamata reptiles we analysed whole-genome data from 63 species estimating effective population size (N_e) through time using Pairwise Sequentially Markovian Coalescent (PSMC). We then implemented environmental niche modelling (ENM) to reconstruct past suitable habitat dynamics and assess their impact on demographic trends. Our results show recurrent cycles of population expansion and contraction over the last 1 million years across all species. While N_e fluctuations correlated with suitable habitat availability during the Early Holocene, we observed a general decline in population sizes following the Last Glacial Maximum that was not fully explained by environmental modelling alone. To further investigate additional factors involved in these demographic shifts, we performed Bayesian model selection comparing climatic, human presence, and combined. We found that the model identifying increasing anthropogenic influence as the sole primary factor best explained the demographic contraction trend. Finally, using Phylogenetic Generalized Least Squares (PGLS), we tested the influence of life-history traits on genetic parameters and identified a significant correlation between adult body mass and both H and N_e , consistent with patterns observed in other vertebrates. These findings suggest that while intrinsic traits like body size are key drivers of genetic diversity, recent demographic trends in Squamata have been primarily influenced by increasing human impact over time.

Extreme phenotypic divergence within swimming distance: whole-genome insights into insular *Podarcis siculus*

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Island systems have long been recognised as privileged settings for studying evolutionary processes, as drift, founder effects and natural selection can drive rapid phenotypic divergence in small populations. Yet some of the most intriguing cases arise when divergence occurs despite little or no geographic isolation, challenging our understanding of how extreme phenotypes originate and persist in the face of potential connectivity with source populations. We investigated this phenomenon in two Sicilian islets hosting populations of the Italian wall lizard *Podarcis siculus* with coloration that differ markedly from nearby mainland counterparts. The population of Isola Bella, connected to the mainland at low tide by a narrow sand strip. Despite this partial connectivity, it harbours an extreme phenotype. The population on Isola Lachea on the other hand, harbours individuals with a phenotype intermediate of that found on Isola Bella and the mainland. We combined standardized field photography, ddRAD and whole-genome sequencing to investigate phenotypic and genomic variation across the two insular populations and multiple mainland localities. We quantified colour variation, assessed population structure and phylogenetic relationships, estimated genetic diversity through heterozygosity and runs of homozygosity, inferred historical demography, and tested for gene flow among populations. By comparing two insular populations that differ in both geographic setting and phenotypic divergence, this study provides a natural framework to explore the evolutionary processes underlying the origin and maintenance of an extreme phenotype in the absence of isolation.

The Cost of Being Pink: Translating an Evolutionary Trade-Off into a Conservation Strategy for the Galápagos Pink Land Iguana (*Conolophus marthae*)

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While the Galápagos marine iguana (*Amblyrhynchus cristatus*) relies on a thick, melanophore-rich epidermis to survive its harsh marine environment, the critically endangered pink land iguana (*Conolophus marthae*) exhibits a radical evolutionary departure that shapes its survival through a severe biological trade-off. The unique coloration of *C. marthae* is caused by a complete lack of pigmentary cells and a high density of dermal capillaries, exposing its tissues to extreme UVB radiation at Wolf Volcano (up to 600 $\mu\text{W}/\text{cm}^2$). This physiological vulnerability triggers a costly metabolic reallocation: deep-penetrating UVB rays damage DNA and shorten the lifespan of mature red blood cells, forcing the iguana to divert massive amounts of energy toward active, regenerative hemopoiesis. This investment might leave fewer resources for growth and reproduction, resulting in markedly smaller body and clutch sizes than in the coexisting, fully pigmented common land iguana (*C. subcristatus*). Despite these severe physiological costs, this unique morphology is not an evolutionary accident. Recent preliminary genomic investigations have pinpointed positively selected genes involved in the ontogenetic formation of chromatophores in the pink iguana. This counterintuitive adaptation drove a behavioral and ecological shift: to mitigate UVB damage, the pink iguana altered its basking behavior, preferentially selecting sheltered, shaded microhabitats. This niche shift successfully reduced direct competition with the historically dominant *C. subcristatus*, allowing the pink morphology to persist while reinforcing strict behavioral and physiological reproductive barriers that completely interrupt modern gene flow. Translating this evolutionary trade-off into a conservation strategy requires a specific shift from broad macro-landscape management to the strict protection of the shaded microhabitats and specific vegetation canopies that *C. marthae* relies on.

Evolutionary Pathways of Semiochemical Adaptations across Cuckoo Bee Clades

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Brood parasitism is a specialized form of kleptoparasitism in which parasites lay eggs in the nests of host species, which then provide shelter and provision for the parasite's offspring. In bees (Anthophila), this habit has evolved at least 15 times independently, accounting for approximately 13% of species globally (~2,600). Phylogenetic relationships within the parasite-host dyad vary considerably, ranging from parasites following a "loose" version of Emery's Rule (e.g., taxonomic proximity within the tribe Megachilini) to lineages exploiting hosts from different families (e.g., the tribe Nomadini). This diversity makes bees an ideal model for investigating the parallel evolution of parasitic adaptations. Brood-parasitic species of solitary bees, often referred to as cuckoo bees, are far more numerous and phylogenetically diverse than those of social bees. Yet, our current understanding of adaptations to kleptoparasitism is predominantly based on social parasites. Strategies such as chemical mimicry (active imitation of cuticular and glandular recognition cues), chemical camouflage (acquisition of host pheromones), or chemical insignificance (reduction of detectable cues) are well-documented in parasites of eusocial Hymenoptera. Although similar semiochemical strategies may also be adaptive for parasites of solitary bees, they remain poorly understood. In this contribution, we report the results of our recent studies on brood parasite-host dyads belonging to the same and to different families (*Melecta-Anthophora*, *Melecta-Habropoda* in Apidae; *Coelioxys-Megachile*, *Stelis-Megachile* in Megachilidae; *Sphecodes-Lasioglossum* in Halictidae; *Nomada-Andrena* in Apidae and Andrenidae), to clarify whether semiochemical strategies are employed, and of what type, by solitary cuckoo bees of different clades.

Niche breadth and dispersal in spiders: a comparative landscape-genomics approach across 13 co-distributed species from the Canary Islands.

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Understanding the relationship between niche breadth and dispersal remains a central open question in biogeography, with implications for evolutionary biology and macroecology. While classical theory predicts high dispersal for generalist species, empirical studies have repeatedly shown a counterintuitive pattern, with specialists displaying higher dispersal. Methodological constraints related to traits, organisms and analytical limitations have so far obscured the evolutionary pattern. The Canary Islands offer a suitable system to address this question, with sharp ecological transitions between the closed humid habitat of laurel forest (Laurisilva) and the open xeric thermophilous scrubland (dominated by *Euphorbia*) occurring over short geographic distances. We address this question with 13 phylogenetically diverse spider species, co-distributed across an East-West gradient in Tenerife and sampled across a shared set of locations. Species were classified as habitat generalists (occurring in both Laurisilva and *Euphorbia* scrubland) or specialists (restricted to one habitat type). All the species disperse by ballooning, allowing us to hold dispersal mode constant and directly contrast specialists and generalists. Using ddRAD-derived genome-wide SNPs, we directly test whether habitat specialists show lower realised dispersal than generalists, estimating species-specific isolation-by-distance (IBD) slopes from pairwise individual relatedness at multiple spatial scales. This test is embedded in a broader population genomic characterization (diversity, *F_{st}*, isolation by environment, scans for habitat-associated loci) providing the ecological

and adaptive context. This work represents a comprehensive, cross-species test of whether habitat specialization is associated with reduced dispersal and stronger local adaptation in an insular arthropod community, and highlights the power of such direct comparative approaches within tractable insular settings.

Bet-hedging and life cycle diversification in *Eucypris virens* (Crustacea:Ostracoda) facing unpredictable environments

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Dormancy represents a key life history trait in organisms inhabiting temporally variable environments, acting as a bet-hedging strategy that buffers fitness against environmental unpredictability. In the freshwater ostracod *Eucypris virens*, photoperiod mediated plasticity enables alternative life cycle strategies, ranging from univoltine to bivoltine cycles, through differential allocation to resting and nonresting eggs. Experimental evidence shows that dormancy not only allows escape from adverse conditions, but also enhances future reproductive performance, while repeated dormancy deprivation reduces fecundity. Here, we develop a stochastic modelling framework to investigate the evolutionary dynamics of univoltine and bivoltine life cycles under variable hydroperiod regimes. The model is based on an egg bank approach incorporating resting egg hatching, deterioration, and production, with environmental stochasticity introduced through variation in hydroperiod occurrence and duration. Photoperiod functions as a cue linking environmental predictability to life cycle expression. Univoltinism is modelled as a single reproductive generation producing resting eggs, whereas bivoltinism includes an additional within season generation when hydroperiod length permits. Life history parameters are constrained using experimental data, including fitness costs associated with dormancy deprivation. This framework provides an evolutionary perspective on when bivoltinism constitutes an adaptive strategy and when it may become maladaptive under increasing environmental unpredictability, such as that expected under climate change scenarios with warmer and drier winters in Northern Italy.

Eco-evolutionary dynamics of range expansion: how genomic and ecological factors drive colonisation success and failure in marine snail

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Understanding why some populations successfully colonize new environments while others fail is a central question in ecology and evolution, with urgent relevance under ongoing global change. We address this question using the marine snail *Littorina saxatilis*, a uniquely tractable system exhibiting repeated natural colonization events — both successful and failed — across ecologically diverse environments. This species offers a rare opportunity to study the reciprocal interactions between ecological context and evolutionary trajectories that determine whether populations persist, adapt, or go extinct at range margins. Our study integrates 380 newly sequenced samples from previously unstudied areas, including introduced

populations, with 420 existing genomes (800 individuals total). Combining whole-genome resequencing at 10x, phenotypic trait variation, demographic modelling, and fine-scale environmental data, we investigate: (1) how diversity loss, genetic load, and local adaptation shape colonization outcomes; (2) how demographic history, genomic architecture, and admixture modulate selection efficiency; and (3) whether evolutionary background and adaptive traits predict colonization success. We also characterize a poorly understood ecotype associated with brackish environments, shedding light on ecological specialization at range margins. This work contributes to the GENOA (Genetic Nature Observation and Action) Cost Action activities of building pan-European genomic databases, standardizing biodiversity monitoring protocols, and linking genetic variation to ecological change. By bridging population genomics, evolutionary ecology, and conservation biology, our findings advance our understanding of eco-evolutionary mechanisms governing biodiversity dynamics in a changing world.

Ecotype-specific salivary gland expression associated with trophic specialization in the corallivorous gastropod *Coralliophila meyendorffii*

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Several gastropod lineages have evolved the ability to feed on cnidarians despite their venomous defenses; however, the molecular basis of this adaptation remains poorly understood. *Coralliophila meyendorffii*, belonging to the muricid subfamily Coralliophilinae, feeds on multiple anthozoan hosts, including sea anemones, scleractinians, and zoantharians, providing a suitable system to investigate the relationship between gene expression and trophic association. To understand the molecular basis of trophic adaptation in *C. meyendorffii*, we carried out transcriptomic and proteomic analyses on two functionally different foregut glands: the salivary glands (discharging their secretion directly on the host) and the gland of Leiblein (with a secretion confined in the digestive tube). We characterized tissue-specific gene expression in two sympatric ecotypes of *C. meyendorffii* associated respectively with the scleractinian *Astroides calycularis* and the zoanthid *Parazoanthus axinellae*, representing two divergent anthozoan lineages. Further analyses focused on the effects of host-switch on salivary glands' gene expression, integrating miRNA, transcriptomic, and proteomic data, to investigate regulatory mechanisms underlying trophic specialization at the molecular level. Our results highlighted a conserved expression profile across ecotypes for the gland of Leiblein, congruent with a general digestive function, whereas salivary glands exhibited strong ecotype-specific divergence, in line with their key role in host interaction. Host-switch experiments highlighted the presence of both transcriptional and post-transcriptional regulatory mechanisms that allow the snail to exploit different hosts. Host association in this corallivorous snail seems to be driven by short-term regulatory changes in gene

TALKS - Symposium 5: “The evolution of ecological diversification: mechanisms,
interactions, and outcomes”

expression, suggesting that *C. meyendorffi* could be a suitable model system for studying phenotypic plasticity and ecological adaptation in marine environments.

Visual adaptation in deep-sea octopods: compensatory evolution of rhodopsin in a single-opsin visual system

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Octopods possess highly developed camera-type eyes and rely primarily on a single visual opsin, rhodopsin (r-opsin1), for image-forming vision. Despite this apparently constrained visual system, octopods have successfully colonized environments ranging from shallow coastal waters to deep mesopelagic habitats characterized by low light intensity and blue-green dominated wavelengths. How visual adaptation occurs in such a single-opsin system remains poorly understood. Here, we investigated the molecular evolution of r-opsin1 in seven octopod species from the Gulf of Naples spanning littoral and deep-sea habitats. Transcriptomic analyses of retina and optic lobe tissues confirmed that r-opsin1 is the predominant opsin expressed across all species examined. Positive selection analyses did not reveal a general adaptive signal associated with habitat depth, but identified lineage-specific signatures of selection in the mesopelagic species *Pteroctopus tetracirrhus*. Structural modelling showed that several selected residues are located near the retinal-binding pocket. Functional in vitro assays demonstrated that two amino acid substitutions, I87V and F201N, strongly alter rhodopsin spectral properties when introduced individually, whereas the double mutant restores a wild-type-like absorption spectrum, suggesting compensatory epistatic interactions that stabilize protein function. Despite ecological divergence, both shallow- and deep-water octopods retained peak spectral sensitivities within the blue-green range (~480 nm). Homologous mutations were also identified in unrelated deep-sea octopods from different oceanic regions, supporting convergent molecular trajectories associated with low-light environments.

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interactions, and outcomes”

Together, our results show that visual adaptation in octopods can occur through targeted molecular changes and compensatory interactions within a single-opsin visual system, without requiring opsin diversification.



I think



Symposium 6: “Evolutionary biology without borders”

Chairs: Alessio Iannucci, Sara Fratini



The evolution in locomotion adaptations in the *Lynx* lineage: a geometric morphometrics approach

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European extant species of the genus *Lynx* are today highly threatened by anthropogenic pressure. Specifically, *Lynx pardinus* is one of the most endangered carnivoran species in the world, with a fragmented and reduced distribution and constant threats by human pressure. Furthermore, this genus represents one of the apex predators in the ecosystems where it is found, thus its conservation is fundamental to preserve the balance of such natural systems. Comprehending ecological potential and past geographical distribution of this genus might represent an advantage in present and future conservation programs, helping the survival of these animals. In this view, the study of fossil material can help in better shaping the ecological potentialities and former distribution of lynx species. Particularly, the comprehension of locomotion adaptations and predatory behavior can shed light on the habitat distribution and hunting capabilities that this genus can/could occupy. We have thus focused our analysis on postcranial specimens of the possible ancestor of *L. pardinus* (i.e., *Lynx issiodorensis*) to better comprehend its locomotion adaptations. Specifically, we have analyzed the postcranial material of *L. issiodorensis* stored in the Paleontology Museum of Florence and coming from the Upper Valdarno Basin (Tuscany, Italy) to assess the past ecological niche breadth of this species and thus comprehending past adaptations of this genus in a context with no human pressure. Our geometric morphometrics approach, focused on the hindlimb of this species, showed how this genus was adapted to a more scansorial lifestyle, thus suggesting that the main terrestrial adaptations of this genus are a recent acquisition, possibly linked to ecological competition with other more adapted to a scansorial life felids (e.g., *Felis silvestris*). Indeed, when *L. issiodorensis* thrived, no other scansorial carnivoran was present in Europe and it is plausible that this species filled this ecological niche.

The mixed flavour of the herpetofauna of Madagascar

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The island country of Madagascar contains a large and old (ca. 85 My) continental island and numerous smaller peripheral islands. Despite the limited fossil record, it is thought that most of its Cretaceous herpetofauna went extinct during the last mass extinction (66 Mya), at the boundary between the Cretaceous and Paleogene (K-Pg). Time-calibrated phylogenies suggest that some old lineages of vicariance origin are present (e.g. *Erymnochelys madagascariensis*), but are few, and most of its extant herpetofauna is the result of species diversification processes that took place within the island. These started from a limited number of unrelated colonizers that reached Madagascar by rafting along favorable oceanic currents, mostly from Africa, after the K-Pg mass extinction. During these events, Madagascar was already an isolated landmass and its biota had likely remained heavily depauperated. The colonizers that arrived subsequently thus had access to a vast ecospace that enabled them to diversify widely. Looking at present-day species compositions, we observe a correlation between the age of a clade and its species-richness, with the oldest, yet post K-Pg boundary radiations, being the most species-rich. Similarly those radiations that have a large proportion of species diversity in rainforests are significantly more species-rich and have higher micro endemism patterns. Due to their high species richness and ecomorphological diversity, several old clades of Malagasy amphibians and reptiles have been proposed as adaptive radiations. In contrast with this hypothesis, accelerated phenotypic evolution is often associated with constant diversification rates (i.e. Mantellid frogs, ca 61 My; Pseudoxyrhophiinae snakes, ca. 37 My; Scincinae skinks, ca. 58 My), which can possibly be explained by their ancient origin. On the contrary, younger clades show a more conserved morphology (e.g. Hyperoliid frogs, *Astrochelys-Pyxis* tortoises), lower species-richness and, in some cases, wider distribution.

A new phased assembly of the Antarctic spiny plunderfish provides novel insights into the evolution of the notothenioid radiation

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How species adapt to extreme environments is a fundamental question in evolutionary biology. Notothenioids are a well characterised species flock endemic to the Antarctic and an important model group for the study of genome adaptation to extreme cold. Here, we used a new haplotype-resolved reference assembly of the notothenioid *Harpagifer antarcticus* and clade-wide comparative genomic analysis to investigate notothenioid evolution and the appearance of novel functionalities linked to cold adaptation. Comparison of the two haplotypes revealed low levels of variability across the genome, nevertheless, numerous insertions from multiple ancient LINE-L2 lineages were found, suggesting ongoing transposition with potential contribution to speciation. Contrary to expectations, the antifreeze locus was highly similar between haplotypes, except for large length allelic variants of *afgp* genes. Structural and phylogenetic analyses suggest a model for the expansion of the locus through segmental tandem duplications involving two pairs of *afgp* genes at time. Comparison of chromosome-scale genomes from across the clade demonstrates conserved macrosyntentic relationships and group specific chromosomal fusions. Despite this genomic stability, cryonotothenioid experienced a first ancestral slow genome expansion followed by lineage-specific massive peaks of genomic gain and transposition activity, concurrently with historic temperature drops. Finally, we identified a set of genes that underwent ancestral diversifying selection and acquired novel conserved non-coding elements during the cryonotothenioid emergence. These genes were related to antioxidants and proteostasis, two processes that are important for cold adaptation. Our results show how both diversifying selection and genomic gain linked to transposon activity have contributed to lineage-specific

evolutionary dynamics through the clade potentially facilitating adaptation to life in the cold.

From genes to fiber: uncovering the molecular and biomechanical basis of silk production in the webspinner *Embia thyrenica*.

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Human societies have long drawn on nature's resources, obtaining materials that have transformed cultures and economies. Among these, lepidopteran silk has become a cornerstone of the global textile industry, while spider silk has more recently attracted scientific attention for its remarkable mechanical properties, with applications spanning regenerative medicine, biomaterials development, and drug delivery. Yet silk-like materials have evolved independently across multiple animal lineages, most of which remain largely unexplored - representing an exciting avenue for the discovery of novel bioinspired materials. One such overlooked group is the webspinners (order Embioptera), insects that produce silk from specialized glands housed within the enlarged basitarsomeres of their forelegs. Despite their unique biology, the molecular mechanisms and evolutionary origins of embiopteran silk remain poorly understood. We present an integrative, interdisciplinary study of silk

production in *Embia thyrrenica*, a Mediterranean endemic. We established laboratory colonies of *E. thyrrenica* and generated a high-quality reference genome using long-read and long-range sequencing technologies. We then combined spatially resolved transcriptomic to identify the cellular and molecular machinery underlying fiber synthesis. To characterize gland morphology, we used scanning electron microscopy and X-ray nanotomography to reconstruct three-dimensional models of the basitarsomere silk glands. Finally, we coupled metabolomic and proteomic analyses with tensile testing to quantify the chemical composition and mechanical properties of the silk fibers. By integrating molecular, cellular, and biomechanical scales, our analyses reveal how novel levels of biological organization emerge over evolutionary time. This framework not only illuminates the developmental and evolutionary origins of embiopteran silk but is also expected to establish design principles for next-generation bioinspired materials.

Beyond the shell: genomic divergence between colour morphs reveals immune-related adaptation in Adriatic Pacific oysters

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Magallana gigas (Pacific oyster) is an economically important aquaculture species that is widely farmed around the world. In Italy, although the sector is still developing, it is generating increasing interest. Pacific oysters naturally show variation in shell colour—a commercially valuable trait—with gold and black phenotypes observed in the Sacca di Goro area alongside the common wild type. To investigate the genetic traits involved in local adaptation, the genomes of 10 individuals per shell colour phenotype were sequenced. The sequencing produced a total of 3.45 billion reads, with an average coverage above 20× and a mean quality score of 35. Variant calling identified approximately 14 million polymorphisms across the three subgroups. Population structure analysis revealed a substructure based on shell colour, consistent with a recent (~60 years) colonization of the Upper Adriatic by *M. gigas*. Fst analysis indicated higher genetic differentiation between the black and gold morphs and led to the identification of 377 genomic regions potentially under selection. Gene Ontology (GO) enrichment and functional analyses of these regions revealed 307 genes associated with 36 significantly enriched biological processes ($p < 0.05$). Notably, the dataset showed enrichment for GO terms involved in immune response regulation, leukocytes activation and protein modification related to protein degradation or cellular signalling.

A congruent solution to mosquito phylogeny based on multiple genome-scale datasets and analytical conditions

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Nematocerans (mosquitoes, midges, gnats and their allies) play key ecological roles as nutrient recyclers and as vectors of human and animal diseases. Yet our understanding of their evolutionary history remains controversial, because of recurrent phylogenetic inconsistency among studies and datasets. To investigate this conflict, we assembled and analysed the largest phylogenomic, mitogenomic, and ultraconserved-element (UCE) datasets to date for the group. To ensure compositional stability, we sampled 86 Culicidae and 80 outgroups spanning Culicoidea, Chironomidae, Psychodomorpha, Ptychopteridae, Anisopodidae, Bibionomorpha, and Brachycera. We recover overwhelming support for the monophyly of Culicinae and a robust backbone for Culicomorpha and the early diversification of other nematocerans. Divergence-time estimates reaffirm an ancient Cretaceous origin of mosquitoes and a Triassic diversification of the main nematoceran lineages. We probed the stability of the phylogenetic signal across all three datasets under a range of analytical conditions, testing the effects of taxon sampling, inference method, substitution model, and compositional bias. Results indicate that previous evidence for a paraphyletic Culicinae from UCE data is a compositional artefact. The concision of nuclear genes, UCES, and mitochondrial markers yields a robust hypothesis for the relationships and timeline of mosquitoes and other nematoceran lineages, offering a critical framework for future comparative work on trait evolution, biogeography, and vector biology. Methodologically, our results reaffirm a foundational principle of phylogenetic inference: dense, balanced, and thoughtful outgroup sampling is the foundation on which deep phylogenies should be tested.

Evolutionary drivers of transposable elements accumulation in swallowtail butterflies and passerine birds

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Genome size is a widely variable trait across the tree of life. While transposable elements (TEs) are known to be pivotal determinants of genome size, the evolutionary mechanisms underlying their proliferation or reduction in different lineages - and ultimately genome inflation or downsizing - are not well understood. Because TEs may disrupt genomic integrity, they are considered as mostly neutral or deleterious to their host. The drift barrier hypothesis postulates that the removal of TEs is as effective as purifying selection, which is stronger in species with larger effective population sizes (N_e). Hence, larger genomes are expected when N_e is small due to the random fixation of detrimental TEs. Here, we study the impact of genetic drift on genome size variation and TE accumulation on short evolutionary times using two independent datasets with contrasted patterns of genome size or N_e , composed of 33 Papilionidae and 76 Passeriformes species. In spite of the 8-fold genome size variation in Papilionidae, we fail to find an association between genetic drift indices (dN/dS , heterozygosity) and genome size, overall or recently accumulated TE content. Interestingly, larger genomes show more frequent outgoing TE lateral transfers, suggesting that while TE-rich genomes more readily spread TEs to other species, this process does not drive genome size expansion either. While more homogeneous in genome size, Passeriformes exhibit multiple transitions from mainland to insular lifestyle, providing contrasted N_e . No link between relaxed selection and genome size is observed in birds either. However, species undergoing stronger drift accumulate more TEs and notably display higher loads of full-length, young elements. Our findings are consistent with a nearly-neutral accumulation of TEs in the short term in birds, as predicted by the drift barrier hypothesis. However, this does not translate into longer-term differences in genome size, nor is it a consistent trend across taxa.

The neurosecretory brain-gut axis evolution and function: from regulome and cell identity to cellular crosstalk

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Organismal survival relies on the ability to interact with the environment and digest food. These traits, linked by neurons, immune system elements, and hormones, regulate physiological and developmental processes, establishing the regulatory axis termed the “brain–gut axis.” Vertebrate neurosecretory brain centers in the hypothalamus predate the origin of vertebrates, with neuroendocrine control mechanisms observed in all animals equipped with a nervous system, suggesting an ancient origin of bilaterian brains. Although it is widely acknowledged that the brain–gut axis appeared very early in animal evolution, questions such as when the brain–gut axis evolved and the origin of neuroendocrine functions remain unknown. To study this, we use echinoderms, non-chordate deuterostomes with an enigmatic nervous system and a wide variety of neuroendocrine cell types, including pancreatic-like ones that predate the emergence of the pancreas as a distinct organ. In particular, we use sea urchin larvae and juveniles, which represent diversified body plans and lifestyles, planktonic and benthic, respectively, and can feed and grow while interacting with the environment. This feature offers an almost unique experimental system to disentangle causality from genotype to phenotype at the molecular, cellular, morphological, and gene regulatory network levels, in connection with environmental inputs and the organism as a whole. Within this project, we are building multimodal integrated atlases spanning the single-cell transcriptome, regulome, volume electron microscopy reconstruction, and connectome of sea urchin cells. Focusing on pancreatic endocrine cells, as well as to all neuroendocrine cell types connecting brain and gut, we are reconstructing the complete neurosecretory apparatus controlling the brain–gut axis in echinoderms. Finally, using comparative transcriptomics, we investigate the origin and evolution of the brain–gut axis across metazoans.

Molecular evolution of hexamerins-encoding genes (storage proteins) in pollen-feeding *Heliconius* species (Nymphalidae)

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Dietary adaptation represents one of the major selective forces driving evolutionary divergence in insects. *Heliconius* butterflies represent a remarkable case: unlike most Lepidoptera, they have adapted to actively collect and consume pollen, gaining access to amino acid resources throughout their entire life cycle. This innovation supports extended lifespan, sustained reproduction, and investment in energetically costly traits such as chemical defences, raising the question of how existing molecular systems sustain this shift. At the physiological level, storage proteins (hexamerins) play a crucial role in accumulating amino acid resources during the larval development, acting as a reservoir for metamorphosis and adult sustenance. To address the potential role of storage protein genes in supporting pollen feeding in *Heliconius*, here we traced the evolutionary history of hexamerin-encoding genes across 75 Nymphalidae species, comparing them to phenoloxidasases, a more conserved protein family primarily involved in immunity. Across 795 genes grouped into nine orthologous groups, we identified lineage-specific duplications and divergent evolutionary trajectories associated with *Heliconius*. Selection analysis revealed signatures of both relaxed and positive selection, consistent with a combination of functional constraint release and adaptive diversification. To address how these changes are reflected into the protein architecture, we predicted 525 complete protein structures and analysed their structural relationships, and mapped sites under selection, tracing the enrichment and depletion shifts of essential amino acids, including residues actively involved in toxin biosynthesis in Heliconiinae. Together, these results suggest that an ancestral storage system has been at least partially reshaped in the context of dietary innovation, providing a mechanistic glimpse into

how ecological novelty can be supported by the evolutionary remodeling of existing genetic tools.



I think



POSTERS



The Obscure Blend of Phenotypic Drivers: A Multi-omics Integrative Framework in Springtails

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Host microbiome research has been expanding, acknowledging its potential contribution to arthropod phenotypic traits including stress tolerance. However, the role of microbiome in springtails remains understudied. Here, we investigate 23 *Entomobrya nicoleti* populations sampled across Danish grasslands. We examine how their microbial composition, host genomic variation and environmental parameters may contribute to host heat, cold, and fungal infection tolerance. We combine short-read metagenomic analysis with assembly-based approaches to identify associated microbial taxa, assess their potential function and association with phenotypic differences across populations. In parallel we analyze host genomic variation to identify potential population signatures of adaptation. Environmental data for sampled sites provide an ecological context to understand the relationship between host and microbial variation with habitat conditions. These include soil pH, temperature, dissolved oxygen and nitrogen content. Combining these datasets, we will use redundancy analysis (RDA) and multi-omics factor analysis (MOFA) to explore the shared and independent contributions of microbiome, host genetics and environmental parameters to stress tolerance traits. This generates candidate host-microbe interactions for future experimental validation. By combining ecological, genomic, and microbiome, this study aims to clarify how microbial and host variation jointly shape adaptation in a soil-dwelling arthropod system.

Do bacterial symbionts manipulate unicellular eukaryotic hosts? A perspective on the evolution of reproductive parasitism

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Bacteria and eukaryotes engage in complex and variegated interactions. The noteworthy *Wolbachia* is able to influence the reproduction of arthropod hosts to favour its own transmission by various mechanisms, including cytoplasmic incompatibility. This is realised thanks to a dedicated molecular machinery, derived from prophages and analogous to toxin-antitoxin systems. More recently, several other phylogenetically diverse bacteria were shown to display comparable capabilities to manipulate the reproduction of arthropods. Here we wonder whether bacterial symbiont may also manipulate other non-arthropod hosts, particularly protists, which represent primary models for evolutionary investigations on eukaryotes, due to their diversity, ecological diversification, and antiquity, but are still under-investigated. Therefore, we performed a thorough literature review and re-interpretation on bacterial-protist interactions with this question in mind. Accordingly, we conclude that such bacterial “addictive manipulators” of protists do exist, and exploit dynamics analogous to those in arthropods but with significant differences, due to significant variations in host physiology and life cycles. Manipulators of protists might be quite common but until now overlooked in non-targeted investigations, considering their elusive features. We posit that the recurrent evolutionary repurposing of bacterial toxin-antitoxin systems might be crucial in the addictive manipulation of protists, which suggests intriguing functional analogy and molecular homology with plasmid-bacterial interactions. We also noticed that multiple of such addictive manipulators are affiliated to bacterial lineages which display ancient associations with diverse eukaryotes (e.g., *Rickettsiales*, *Legionellales*), suggesting that addictive manipulation of protists might have paved the way to the evolution of bacteria associated with multicellular organisms, including pathogens.

Integration of epigenetic and transcriptomic strain-specific variations suggests the presence of unexplored layers of adaptive evolution in bacteria

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Epigenetic variation is increasingly recognized as a key source of heritable phenotypic diversity in bacteria, driving rapid adaptation and long-term evolutionary change. DNA methylation can generate stable, transmissible differences in gene expression among genetically identical cells, contributing to strain-specific ecological strategies. Characterizing these landscapes at a genome-wide scale is essential to understand how bacterial populations explore adaptive space. *Sinorhizobium meliloti* represents an ideal model for investigating these processes. Its complex multipartite genome and ability to colonize radically diverse ecological niches make it a powerful system for studying how epigenetic modifications contribute to ecological diversification and adaptation. Here, we present a framework for the characterization of these landscapes by integrating methylomic and transcriptomic data. We implemented a bioinformatics workflow tailored for high-throughput PacBio Revio sequencing, to achieve rigorous quality control of both adenine and cytosine methylation counts. These profiles were coupled with RNA-seq data from cultures grown under identical conditions. This multiomic approach allows us to partition sources of transcriptional variation - genetic, epigenetic and environmental – and to evaluate whether these epigenetic states are heritable, environment-responsive, or both. Building on this high-resolution data, we assess genome-wide methylation and transcriptional divergence across strains and environmental conditions, combining motif analysis with functional and positional mapping. Preliminary results from four *S. meliloti* strains exposed to sub-inhibitory NaCl concentrations indicate co-variability between methylation patterns and gene expression, supporting a potential role for epigenomic regulation in strain-specific adaptive responses.

Ingestion of biopolymers by talitrus saltator: effects on the gut morphology and the microbiome

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Despite the promise of a reduced environmental impact, bioplastics are subjected to dispersion and accumulation similarly to traditional plastics, especially in marine and coastal environments. While the supralittoral amphipod *Talitrus saltator* is known to ingest and survive on starch-based bioplastics, the precise role of its gut microbiota in processing these materials remains poorly understood. This study investigated how bioplastic consumption reshapes the *T. saltator* gut microbiome and whether these microbial shifts facilitate the chemical breakdown of the ingested polymers. Adult amphipods were maintained on distinct dietary regimes: two types of starch-based bioplastics, a 50/50 chitosan-starch mixture, or a control diet of paper and dry fish food. Freshly collected, unfed individuals were used as control group. Fecal pellets were characterized using ATR-FTIR spectroscopy, and gut microbial DNA was analyzed via metagenomic sequencing. Spectroscopic analysis revealed the partial digestion of polysaccharide components in the experimental polymeric materials. Concurrently, metagenomic profiling demonstrated that bioplastic ingestion significantly restructured the sandhopper's gut microbial communities. This shift was marked by an enriched abundance of specific bacterial genera associated with plastic and bioplastic biodegradation, a phenomenon most pronounced in the groups sustained on starch-based bioplastics. Collectively, these findings demonstrate the active involvement of the *T. saltator* gut microbiota in biopolymer modification. These results provide critical insights into the functional ecology of coastal detritivores and highlight the potential of sandhopper-associated microbial consortia for targeted bioplastic waste management and bioremediation strategies.

Ecological, functional, and evolutionary characterization of the microbiota of spontaneously fermented meat using shotgun metagenomics

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Fermented meats such as salami and ham constitute dynamic microbial ecosystems in which bacterial and yeast communities evolve under selective pressures imposed by meat type, spice composition, and maturation conditions. To address the fragmented state of current knowledge, we first conducted a global survey of 243 fermented meat samples reported across 165 studies employing three methodological approaches (culture-dependent isolation, amplicon sequencing, and metagenomics). Across 1,086 detected taxa, no conserved pan-microbiome emerged; nevertheless, twenty-five bacterial taxa were identified in at least one sample from each major product category (ham, sausage, salami, meat, and other fermented products). Methodological approach explained microbial diversity patterns more strongly than either geographical origin or product type. We then performed shotgun metagenomic analyses on 51 spontaneously fermented meat samples collected worldwide, including six newly sequenced Italian Ciauscolo-style salami from three areas of the Marche-Umbria region. *Latilactobacillus sakei* dominated nearly all minced-pork products, together with *Latilactobacillus curvatus*, *Lactococcus lactis*, *Staphylococcus equorum*, and the yeast *Debaryomyces hansenii*. Strain-level phylogenetic reconstruction of *L. sakei* revealed marked biogeographical structuring at establishment, regional, and national scales. Building on these findings, our long-term objective is to generate the first time calibrated phylogeny of *L. sakei* integrated with genome-wide selection analyses, in order to clarify when and how this key component of the cured-meat microbiota became associated with human food production whether during the Neolithic transition or more recently alongside nineteenth-century industrialisation.

More than meets the eye: unraveling anthropic land use impacts on skin microbiota of an opportunistic amphibian species

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As amphibians continue to register population declines worldwide due to human-driven habitat modifications and emerging diseases, their skin microbiota has attracted major interest as both a possible means of adaptation to the changing environment and a barrier against pathogens. Here we focussed on *Bombina variegata*, a small anuran colonizing both natural and artificial water bodies, to investigate differences in skin microbiota composition between individuals living in different habitats across the Province of Trento, Italy. Fourteen populations were sampled, including those of natural ponds, seasonal ponds on agricultural land, water tanks, and farm ponds. Skin and water microbiota were investigated using metataxonomy by targeting the bacterial V3-V4 16S rRNA gene and fungal ITS1 loci. Our results highlight a significant association between skin and water microbiota across all investigated habitats. Composition and diversity of skin microbiota changed between habitats, with the skin microbiota of animals collected from natural ponds being characterized by lower alpha diversity and distinct bacterial and fungal composition. Furthermore, observed variation in skin microbial diversity could be partially explained by the water parameters: pH, dissolved oxygen, and temperature. Implication of human-driven habitat modifications and water composition on *B. variegata* skin microbiota for the conservation of this species are discussed.

Sympatric *Lepus* spp. in the central Italian alps host significantly different gut microbiotas

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The mountain hare (*Lepus timidus*), an arctic–alpine species with relictual populations in the Italian Alps (>2000 m a.s.l.), is threatened by habitat fragmentation and the upward expansion of the European brown hare (*L. europaeus*), risking resource competition and hybridization. The gut microbiota is vital for animal health; therefore, investigating it in species exposed to rapid biotic and abiotic environmental changes may improve our understanding of their conservation status and extinction risk. However, the effects of sympatry on gut microbiota diversity and composition remain largely unexplored. Here, we compared the gut microbiota of these two species within an overlap zone in the central Alps (Val Mazia, Italy) by analyzing fresh fecal pellets collected along an elevational gradient (1000–2500 m a.s.l.). Species identity was confirmed using mtDNA barcoding, while the gut microbiota was characterized via amplicon sequencing (prokaryotic 16S rRNA V3-V4 and fungal ITS2). Distinct prokaryotic and fungal communities were detected for each species, even in areas where their distributions overlapped. Moreover, predicted functional profiles derived from taxonomic composition revealed differences in biosynthetic and metabolic potential between the host-associated microbiotas of the two species. Elevation influenced fungal, but not prokaryotic, diversity in both species. Overall, our findings suggest that sympatry has so far had a limited impact on the gut microbiota composition of either species. Considering climate warming and continued hunting restocking of *L. europaeus*, this study provides an important baseline for assessing the health and adaptability of *L. timidus*, as well as the effectiveness of conservation measures. Extending this research to additional areas of sympatry will be essential to determine whether gut microbiota composition can serve as an indicator of *L. timidus* conservation status across its range.

Multi-marker analysis of the bark beetle mycobiome detects significant region-specific biases of the fungal community

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Fungal symbionts play a fundamental role in shaping diverse insect traits. The advancement of high-throughput amplicon sequencing has enabled the study of large-scale microbial communities independent of organism cultivability. While the 16S rRNA gene remains the “gold standard” for bacterial communities, selecting the optimal genomic region for fungi is more challenging. Fungi also have complex associations with bark beetles ranging from nutritional mutualists and detoxification agents to antagonistic species. To fully resolve these relationships, precise taxonomic identification is essential. However, the choice of target DNA region can introduce significant biases in primer binding, amplification, and reference database coverage, which in turn distort the perceived community structure. To quantify these effects and evaluate existing tools, we compared the three most common ribosomal DNA regions for fungal metabarcoding - the ITS, LSU, and SSU - using samples from eight different bark beetle species. Our results indicate that while the ITS region captures the broadest overall taxonomic diversity, the LSU and SSU provide superior resolution for specific beetle-associated taxa. Marker-specific biases in the detection of rare species resulted in samples clustering by rDNA region rather than host species in PCA and NMDS ordinations. These biases were exemplified by the detection of *Raffaelea* spp. exclusively via SSU, while *Grosmannia* spp. were identified by ITS and LSU. By presenting this multi-marker analysis of the bark beetle mycobiome, we provide a critical benchmark for assessing regional biases in fungal metabarcoding. These findings offer guidance on marker selection for future studies of the mycobiome of insects.

Characterising ecological alterations of the oral microbiota in head and neck cancer patients with sustained or severe acute oral mucositis

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Emerging evidence indicates that the human oral microbiome can influence treatment efficacy and side effects in Head and Neck Cancer (HNC). In particular, due the radiotherapy and cytotoxic agents employed in the standard treatment regimen, the majority of patients develop acute oral mucositis (AOM), a painful inflammation and ulceration of the oral mucosa, and xerostomia ("dry mouth"), with a severe impact in both life quality and survival rates. Here we aim to better understand how the oral microbiome interacts with the development and progression of HCN side effects. We leveraged the data from the MicroLearner observational study (NCT03294122), which includes pre-radiotherapy and post-radiotherapy salivary microbiome matched profiles of 159 HNC patients with associated toxicity and clinical data (e.g. adjuvant therapies, comorbidities, additional pharmacological treatments). Patients' oral microbiome was characterised using both 16S rRNA amplicon and WGS sequencing. We employed a differential co-occurrence analysis to identify ecological interactions between taxa showing significative alteration in patients affected by more severe forms of AOM/xerostomia compared to patients with milder forms. We will use the obtained data to predict which features of the patients microbiome affects the probability of side effects, to identify patients at higher risk.

Functional characterization of ancient OT439 genomes reconstructed from dental calculus

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The transition from hunting-gathering to farming during the Neolithic profoundly shaped human evolution. Archaeological remains have shown that, in this period, humans adopted new ways for processing foods and cooking, highlighting how this shift profoundly impacted not only the evolution of humans but also of their associated microorganisms. Since the microbiome actively shapes host adaptation and health, these ancient microbial communities provide a unique window into past human lifeways. However, there is limited data available on how the human microbiome has changed in composition and function over time. To explore this evolutionary link, we focus on reconstructing the ancient genome of Anaerolineaceae oral taxon 439 (OT439), a bacterium found at higher abundance in the oral microbiome of Neolithic farmers. We focused on the functional annotation of OT439 bacterial genome to detect presence and absence of key genes to investigate how it may have impacted the metabolism of food and medicinal substances across time. To achieve this, we used assembled genomes that present a high level of completeness and low level of contamination to perform the functional annotation using Prokka. To identify homologous gene families, we used BLAST, filtering based on sequence identity and reciprocal coverage thresholds. Filtered BLAST similarities were then used to build a gene similarity network, where genes were grouped into families based on their sequence relationships across samples. A presence/absence matrix of gene families was then constructed to evaluate functional gene distribution and variability among genomes. The results obtained will be crucial to identify specific groups that can be informative about the dynamics of the Neolithic transition, and to formulate evolutionary hypotheses about the contribution of the human oral microbiome to dietary and cultural changes over time.

Plant diets in the Italian Alps: Refining species-level resolution from fecal shotgun sequencing

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Accurate diet characterization is fundamental to understanding the ecology and resilience of wildlife in sensitive alpine ecosystems. While DNA metabarcoding has become a standard tool, it often lacks the taxonomic resolution to distinguish closely related plant species for precise ecological monitoring. We evaluate a high-precision framework based on shotgun sequencing to achieve species-level resolution in plant diet analysis, providing a robust alternative for monitoring mountain wildlife. Our approach is being validated using the Alpine rock ptarmigan (*Lagopus muta helvetica*), a boreal, cold-adapted glacial relict inhabiting high-altitude environments in the Italian Alps, serving as an ideal model for testing dietary resolution across alpine flora. We evaluate shotgun sequencing with the Kraken2 classifier across diverse reference databases, including full genomes, chloroplast markers, and ITS sequences. A key component involves enhancing reference databases by integrating the latest sequences from NCBI-GenBank in a quality-controlled and curated manner, ensuring the highest possible taxonomic coverage for alpine-specific plants. Results highlight the critical role of database selection and demonstrate that shotgun metagenomics allows for parallel identification of plant content and microbial communities within the same fecal samples. Our findings suggest functional links between diet and the gut microbiome; for instance, medicinal plants correlate with reduced pathogenic bacteria, suggesting a role in modulating animal health. Furthermore, detecting soil-health indicators like *Pseudomonas putida* and plant pathogens like *Rhizobium tumorigenes* demonstrates that single fecal samples simultaneously inform on host health, diet, and habitat-level integrity. This scalable approach is highly transferable to the conservation of other alpine species and their habitats, such as those of the Alpine chamois (*Rupicapra rupicapra rupicapra*) or mountain hare (*Lepus timidus*).

Ontogenetic shifts and sex-related evolution of venom system in the spiny dogfish *Squalus acanthias*

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Venom systems have evolved independently multiple times in vertebrates, including across several fish lineages which exhibit substantial morphological diversity in venom delivery structures and biochemical diversity in venom composition. However, the ecological and evolutionary drivers shaping this variation remain largely unexplored. This project investigates how ontogeny and sex influence venom systems in sharks, building based on the hypothesis that selective pressures acting on venom systems shift throughout the life cycle, from predominantly anti-predatory functions in juveniles to additional reproductive functions in adults. In elasmobranchs, juveniles experience high predation risk, whereas sexually mature individuals are exposed to strong reproductive pressures, including male-male competition and aggressive mating behaviours, in which dorsal spines may serve as sexual weapons. Here, we present preliminary results on sex- and age-related variation in the morphology of venomous dorsal spines in the spiny dogfish, *Squalus acanthias*. We also describe the development of a Fourier Transform Infrared Spectroscopy (FTIR)-based

methodological framework to analyse variation in spine composition and structural properties across age classes and sexes. These preliminary findings establish the methodological foundation for the future integration of morphological, histological, proteomic, and functional analyses, which will subsequently be expanded to additional shark species within a comparative framework. This integrative approach will provide the first comprehensive assessment of ontogenetic and sex-related variation in shark venom systems, offering new insights into the ecological and reproductive selective pressures shaping venom evolution in elasmobranchs.

Does biogeographic origin predict cold hardiness? Evolutionary divergence in thermal behaviour among three sympatric *Vespa* hornets

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Biological invasions offer compelling natural experiments for examining how evolutionary history shapes behavioural responses to environmental stress. In Italy, Tuscany hosts three sympatric species of the genus *Vespa*: the temperate native *V. crabro*, the subtropical-origin invasive alien *V. velutina*, and the Mediterranean neo-native *V. orientalis*; each carrying a distinct evolutionary history, while sharing partially overlapping ecological niches. Among the traits potentially shaped by divergent selection pressures, cold hardiness stands out as ecologically critical, as it may simultaneously determine competitive outcomes between native and non-native species and set the physiological limits of range expansion into cooler environments. Within the framework of the ALIEM Vigil project and the Piano di gestione della *Vespa velutina* della Regione Toscana, this study explored behavioural responses to cold exposure (3–4 °C) over two durations (1 and 24 hours), quantifying locomotor inactivation, complete inactivation (chill coma entry), and post-exposure recovery of first movement and full locomotion. The tested workers of the three species exhibited markedly divergent behavioural profiles. *Vespa crabro* entered chill coma earliest and most frequently, yet recovered efficiently after prolonged exposure. *Vespa orientalis* resisted locomotor inactivation longest but recovered full locomotion far more slowly after prolonged cold stress. *Vespa velutina* showed an intermediate and notably plastic profile, combining moderate chill coma resistance with rapid recovery. Each of these profiles is broadly consistent with the species' respective biogeographic history, and together they carry direct implications for understanding coexistence dynamics and predicting range expansion trajectories under ongoing climate change.

Understanding the behavioural integration of a paper wasp social parasite into the dominance network of the host colony

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Social integration, i.e. the incorporation of newcomers into the social structure of a group, is a key process to understand how animal groups form and change. Among insects, obligate social parasites must enter a host colony and exploit brood care to reproduce, representing an outstanding example of the evolution of social integration. To achieve this, parasites evolved complex communicative strategies, but their ability to behaviourally integrate within the host society and the effects at the group level have been overlooked. We propose a framework to assess the behavioural integration of social parasites using a paper wasp model: the host *Polistes dominula* and its obligate social parasite *Polistes sulcifer*. *P. dominula* colonies show behavioural castes determined by a linear dominance hierarchy among foundresses and workers. Only the alpha retains the reproductive role while the others participate in nest maintenance and brood care. *P. sulcifer* enters the colony and assumes the reproductive role by eliminating the alpha. In this scenario, the parasite should integrate into the social network of the host colony and match the alpha's behavioural repertoire. To test this hypothesis, we generated usurped and control colonies and followed inter-individual dominance interactions. We tested whether usurpation influenced social network metrics at global, motif and individual scale. We found an overall success in the integration of the social parasite within the host social network at the levels investigated. Beyond offering insights into strategies adopted by inquiline social parasites, our conceptual and analytical framework can be used to understand social integration across animal societies.

An AI-based image analysis pipeline for automated quantification of boldness: development and validation in a marine snail

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Behaviour plays a key evolutionary role by influencing survival across microhabitats, shaping natural selection on adaptive traits, and contributing to divergence between populations, making behavioural analyses central to evolutionary and conservation biology. Understanding why some populations expand their range while others fail during colonization is particularly relevant in the context of rapid environmental change. Among behavioural traits, boldness—defined as the latency time before an individual moves following a stimulus—is a major driver of range-shift processes,

influencing risk exposure and habitat colonization. Variation in boldness may thus explain range expansion success. The marine snail *Littorina saxatilis* is an ideal model system to study these dynamics due to its pronounced intraspecific behavioural and phenotypic variation and well-documented ecological divergence. Previous protocols for quantifying boldness in this taxon relied on direct visual scoring. To improve repeatability and reduce scorer subjectivity, we introduced video-recorded behavioural assays with three trials per individual. However, the slow and subtle responses of intertidal snails make scoring time-consuming and difficult to standardize, especially for large datasets. To overcome these limitations, we developed an AI-based image analysis pipeline to automatically detect behavioural events and extract boldness measurements in over 1,100 snails from 22 locations across its whole range, including successful and failing colonizations and their sources. The pipeline was validated against manually extracted scores. Our results show that this approach improves efficiency, precision, and repeatability, contributing to understanding behavioural traits in *Littorina* colonization processes. Moreover, it opens avenues for broad application across behavioural studies in diverse taxa, with implications for evolutionary theory and evidence-based conservation under rapid environmental change.

Weighting the evidence: allele frequency strategies for improving LD-based Ne estimation

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Accurate estimation of contemporary effective population size (N_e) is a central requirement for genetic diversity monitoring in conservation genomics. Linkage disequilibrium (LD)-based methods offer a practical single-sample solution, but their performance depends critically on SNP panel composition: rare alleles downwardly bias r^2 and inflate N_e estimates, a problem typically addressed by minor allele frequency (MAF) filtering. Sved et al. (2013) proposed an alternative – weighting locus pairs by allele frequency P or by $P(1-P)$ – since intermediate-frequency alleles carry more information about LD. Waples (2023) further discussed this idea, but both approaches remain unrigorously benchmarked. We evaluated five scenarios differing in how allele frequency information enters LD-based N_e estimation, spanning MAF filtering, preferential SNP selection by P or $P(1-P)$, and pair-level δ^2 -based weighting, as implemented in the software *currentNe* (Santiago et al. 2023). All analyses use exclusively inter-chromosomal SNP pairs ($c = 0.5$), ensuring valid expected r^2 under drift. We used Wright-Fisher forward simulations across a full factorial grid of true N_e , sample size and panel size, evaluating bias, precision and RMSE under each scenario. Results reveal a context-dependent performance trade-off. SNP selection by $P(1-P)$ and P achieve the strongest bias reduction across the parameter space, even at small sample sizes where MAF filtering still shows substantial overestimation. Pair-level weighting provides superior precision at small N_e and low sample sizes, where rare-allele noise dominates variance. RMSE favours different strategies depending on the N_e –sample size regime: pair-level weighting tends to perform best at low S and small N_e , while SNP-selection approaches become more competitive as S and N_e increase. No single strategy universally dominates, and the optimal choice depends on the relative priority of bias versus precision in a given monitoring context.

A pangenomic framework for population genomics and phylogeography of the Mediterranean mussel

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Understanding how genomic diversity is structured across geographic ranges is essential to reconstruct the evolutionary history of a species, understand its adaptation potential and support conservation strategies. This is particularly relevant for marine organisms with large population sizes, high dispersal potential and complex demographic histories, where a single reference genome is often unable to capture the full spectrum of intraspecific variation. Here, we present the first pangenomic resource for the Mediterranean mussel *Mytilus galloprovincialis*. After sampling animals from geographically distinct populations, we assembled six haplotype-resolved chromosome-scale genomes for a total of 12 haplotypes, using PacBio long-read sequencing and Hi-C data. These assemblies were integrated into a comparative pangenomic framework to describe the conserved and variable genomic landscape of the species, including structural diversity and presence-absence variations that may be missed when relying on a single reference genome. As a first application, we mapped whole genome DNA sequencing samples from mussels collected worldwide to test whether the pangenome can improve the recovery of informative markers for population genomic analyses. By reducing reference bias and capturing variants in genomic regions that are absent, divergent or poorly represented in a single genome, this approach aims to provide a more accurate description of population structure and phylogeographic relationships. Overall, this work provides a genomic framework for investigating the evolutionary history of *M. galloprovincialis* and for developing more robust tools in conservation genomics and aquaculture management. More broadly, it highlights the value of pangenomic approaches for non-model species, where hidden structural variation and geographically restricted diversity can strongly influence population genomic inference.

Genetic structure and diversity of Eastern tarsiers

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Tarsiers are nocturnal primates representing an ancient lineage within the haplorhines. They are small-bodied, insectivorous, and produce duet-calls which seem to be species-specific. Morphological differentiation among species is subtle, making genetic data essential for studying their diversification. Sulawesi is a tropical island in Indonesia with a large amount of endemism due to its position in between major biogeographical breaks. Tarsiers are amongst the Asian mammals that have crossed Wallace's Line and colonized Sulawesi, experiencing most of their great radiation within the last 2.5 million years. Here, we used 16 microsatellite loci and 600–1500 SNPs obtained through MiGSeq reduced-representation sequencing of 339 genetic samples from Sulawesi tarsiers to characterize their genetic structure and diversity. SNPs and microsatellites produced largely concordant results. The genetic clusters reflected different evolutionary scales, with the deepest divergence separating the two major genetic lineages previously described for Sulawesi tarsiers, while more subtle clustering patterns were broadly concordant with current species boundaries. However, when accounting for spatial structure, much of the genetic differentiation among species from the Northern Peninsula (*T. wallacei*, *T. supriatnai*, and *T. spectrumgurskyae*), and the high differentiation within *T. dentatus*, was explained by isolation by distance. The genetic diversity decreased towards the tip of the northern Peninsula, which agrees with a history of expansion from the center of the island. We confirm the pattern of hybridization between two species (*T. dentatus* and *T. loriang*) in a contact zone located in central Sulawesi.

Phylogeographically distinct intraspecific lineages of the Western Blacknose Dace, *Rhinichthys obtusus*

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The emergence and maintenance of variation among intraspecific lineages is fundamental to the process of speciation. This is of particular interest in leuciscid fishes, the most diverse group of freshwater fishes in North America. Beyond the extensive species diversity, identifying evolutionarily significant units within these species and understanding the conditions under which they emerge is critical for effective conservation and management. *Rhinichthys obtusus*, the Western Blacknose Dace, offers unique opportunities to examine this process in situ. *R. obtusus* is a North American riffle-dwelling stream minnow with a broad distribution between the Appalachians and the Mississippi River to the east and west, and from the Hudson Bay drainage to the Mobile Bay drainage to the north and south. In this presentation, we will discuss the growing body of evidence for multiple, evolutionarily-distinct lineages within the species through the lens of genetic, genomic, and phylogenomic analyses. We will also explore the biogeographic processes associated with post-glacial recolonization that may explain the current distribution of these lineages. Together, these data enhance our understanding of the considerable diversity observed among leuciscid fishes in North America.

Phylogenomics of the genus *Alectoris*: parapatry, introgression, and speciation in the Palearctic

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Often regarded as an excellent model system for investigating the evolutionary history of Palearctic vertebrates, the genus *Alectoris* comprises seven closely related polytypic species with allopatric or parapatric distributions: the red-legged partridge (*A. rufa*), the rock partridge (*A. graeca*), the chukar partridge (*A. chukar*), the rusty-necklaced partridge (*A. magna*), the Barbary partridge (*A. barbara*), the Arabian partridge (*A. melanocephala*), and Philby's partridge (*A. philbyi*). However, no genomic study has yet comprehensively examined the evolutionary dynamics of the genus as a whole. In this study, we re-sequenced the genomes of 25 *Alectoris* individuals and included re-sequencing data from 10 additional individuals. We recovered more than 3,700 ultraconserved elements (UCEs), identifying 21,706,368 SNPs at a genotyping rate of 94%. Our analyses revealed four major lineages: two basal lineages represented by the Afro-Arabian species *A. melanocephala* and *A. barbara*; a European lineage comprising *A. rufa* and *A. graeca*; and an Asian lineage including *A. chukar*, *A. magna*, and *A. philbyi*. The phylogenetic tree was supported by both concatenation and species tree based approaches. We detected multiple

POSTERS - Symposium 3: "Phylogeography and Conservation Genomics:
understanding evolutionary history to sustain species resilience"

signatures of introgression with notable gene flow between eastern *A. chukar* populations with *A. magna* and *A. philbyi* with other *Alectoris* lineages. While some reflect the intensive management and translocation practices to which several species have been subjected and were therefore expected, others appear to preserve signals of historical connectivity among populations that are currently isolated. This is particularly evident in the case of *A. chukar* and *A. philbyi*, whose genomic patterns suggest past gene flow despite their present-day geographic separation. Comparison of regions of introgression across the genomes identified some areas to be more permeable to gene flow compared to others.

High-altitude survival in a warming world: the case of the White-winged Snowfinch (*Montifringilla nivalis*)

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Mountain species are particularly vulnerable to global warming, which forces them to higher elevations, decreasing suitable habitat and increasing population fragmentation and their level of isolation. The White-winged Snowfinch (*Montifringilla nivalis*) is a high-elevation passerine of the family Passeridae, occurring in Italy across the Alps and Central Apennines. Global warning effects, combined with the strong philopatry and limited dispersal highlighted by previous studies, make assessing the level of population connectivity a key priority for its conservation. We study *M. nivalis* Italian populations by combining contemporary and museum-derived samples. A total of 50 samples from 11 localities across the two mountain ranges were collected, with particular emphasis on Trentino-Alto Adige (32 samples from Eastern Alps, 9 from Western Alps and 9 from Apennines). Whole-genomes were initially used to identify possible differences between levels of genomic variation within areas and to evaluate the degree of population structure. Individual genomic heterozygosity is similar and not particularly low in all samples from the Alps when compared to other bird species (around 0.0012), whereas the Apennine individuals show a 20% reduction. The Alpine and Apennine populations are clearly different, with F_{st} values between 10 and 15%, whereas in the Alps different populations share large fractions of their genomes. The population from Cima D'Asta (Trentino, Alps) shows larger differences between individuals, possibly due to immigration from different breeding areas. These findings contribute to improving our understanding of population connectivity in the species

POSTERS - Symposium 3: "Phylogeography and Conservation Genomics:
understanding evolutionary history to sustain species resilience"

and offer additional insight into the patterns described by previous studies on the partial isolation of Alpine populations. Genomic scans are in progress to investigate the evolutionary potential of this species to adapt to the ongoing environmental change.

"Pseudo-concordance" of phylogeographic patterns among butterflies in the south-eastern Alps

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Sympatric species often show similar phylogeographic patterns, including overlapping transition zones between their intraspecific lineages, or coincident boundaries between their ranges. However, even when transition zones overlap, species may differ in the level of intraspecific differentiation, for instance due to differing colonization histories or biological traits. This "phylogeographic pseudo-concordance" has rarely been documented. To test for the occurrence of pseudo-concordance in a real biological system, we compared four butterfly species (two closely related *Erebia* and two closely related *Pyrgus* species). We focused on a heterogeneous region in the south-eastern Alps, sampling specimens at a spatial density appropriate for the fine-scale landscape structure and to the dispersal ability of these butterflies. We compared differentiation patterns across species embracing a comprehensive perspective on evolutionary divergence including both genomic and phenotypic differentiation. Specifically, we analysed genome-wide SNPs and several morphological characters of wings and copulatory structures. Our results revealed a narrow area encompassing a possible transition zone between two weakly differentiated population groups in both *E. ligea* and *P. malvae*, as well as the boundary between the ranges of two strongly diverging allopatric lineages in *E. euryale*. Notably, although *P. malvoides* does not occur throughout the entire region, its local distribution limit also falls within the same area. Despite the presence of multiple geographic features that could potentially act as barriers, populations on either side of this area did not show additional differentiation. This provides a clear example of pseudo-concordance. Our findings highlight that selecting a complex study area and ensuring spatially dense sampling, and integrating genomic and phenotypic data are effective for detecting pseudo-concordance even in well studied organisms such as butterflies.

Tracing the Tethyan roots of Rissoidae: a global marine micromollusc radiation

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Timing and interplay of diversification, dispersal, and regional persistence, to produce current marine biodiversity remain poorly understood in many small-bodied marine benthic invertebrates. Rissoidae, one of the most diverse families of marine caenogastropods, represent an ideal model to investigate how micromolluscan diversity was assembled through deep time. We reconstructed the most comprehensive phylogenetic framework currently available for Rissoidae, combining mitochondrial and nuclear markers (cox1, 16S, 28S) for 1,289 specimens worldwide, including 807 newly sequenced samples. Using species delimitation, fossil-calibrated divergence-time estimation, and ancestral-range reconstruction, we investigated the temporal and geographic origins of the family and evaluated the evolutionary consistency of traditional shell-based taxonomy. Our analyses recovered Rissoidae as an ancient radiation structured into three major deep lineages, with the earliest divergences dating back to the Jurassic–Early Cretaceous. Ancestral-range reconstruction identified a strong western Tethyan signal in the deeper nodes of the phylogeny, followed by repeated asymmetric dispersal events towards Indo-Pacific and other marine regions. Most diversification events remained concentrated in the northeastern Atlantic, suggesting long-term persistence and regional radiation within

POSTERS - Symposium 3: “Phylogeography and Conservation Genomics:
understanding evolutionary history to sustain species resilience”

the Tethyan–North Atlantic system. Traditional genera were frequently non-monophyletic, highlighting extensive discordance between shell morphology and evolutionary history. These results indicate that present-day rissoid diversity reflects deep Mesozoic origins and long-term biogeographic restructuring rather than predominantly recent diversification. More broadly, our study shows that micromolluscs can retain important macroevolutionary and paleobiogeographic signals, providing valuable insights into the historical assembly of marine biodiversity.

Ancient-modern genomes reveal an impact-dependent erosion of functional diversity in Atlantic bluefin tuna

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Atlantic bluefin tuna (*Thunnus thynnus*) has supported the world's oldest commercial fishery and remains among the most valuable marine resources. Using high-coverage ancient ($n = 34$) and modern ($n = 34$) genomes from the eastern Atlantic, we detect a weak but consistent genome-wide signal of genetic erosion, in line with earlier work and the strong twentieth-century population contraction. Uniquely for a high- N_e marine fish, we quantify temporal changes in putatively deleterious variation using functional-impact proxies. While the genome-wide reduction in diversity is modest, temporal shifts are disproportionately concentrated at functionally constrained sites: modern genomes show a modest depletion at putatively neutral loci but a stronger depletion at variants predicted to be deleterious, scaling with inferred impact and broadly consistent with forward simulations in SLiM. Stratifying allele- and genotype-frequency contrasts by pre-bottleneck derived-allele frequency reveals that, although drift disproportionately affects rarer variants, higher-impact categories still decline more than lower-impact classes at matched starting frequency, supporting an

POSTERS - Symposium 3: "Phylogeography and Conservation Genomics:
understanding evolutionary history to sustain species resilience"

additional role for purifying/background selection beyond drift alone. Together, these results advance empirical baselines for genetic-load dynamics in high- N_e species and reveal an impact-dependent footprint of recent decline on functional genetic diversity, with potential consequences for long-term adaptive capacity.

A chromosome level reference genome of the Broom hare (*Lepus castroviejoi*)

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High-quality reference genomes are essential resources for studying evolutionary processes and supporting conservation efforts, particularly for species with restricted and fragmented distributions. The Broom hare (*Lepus castroviejoi*) is an endemic species of the Cantabrian Mountains (northern Spain), whose limited range makes it of particular interest for evolutionary and conservation genomics. In this study, we present a chromosome-level reference genome assembly for *L. castroviejoi*, generated using a combination of PacBio HiFi long reads and Hi-C sequencing data. The assembly was produced following the pipeline of the Vertebrate Genomes Project, enabling the generation of highly contiguous genomic sequences. Manual curation based on Hi-C contact maps was performed to correct structural inconsistencies and improve chromosomal scaffolding. Quality assessment indicates a high-quality genome assembly, supported by high completeness (BUSCO), strong consensus accuracy (Mercury), and no evidence of significant contamination (BlobToolKit). These results demonstrate the robustness and reliability of the assembled genome. This reference genome represents a valuable resource for future studies on the evolutionary history, population genomics, and conservation of *L. castroviejoi*, providing a foundation for a wide range of possible downstream analyses.

Genetic diversity of the alpine longhorn beetle *Rosalia alpina* within the protected areas network in Italy

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We assessed the extent to which the Italian network of protected areas, comprising national and regional parks and Natura 2000 sites, encompasses regions harbouring the highest genetic diversity of *Rosalia alpina*, a saproxylic longhorn beetle strictly protected under the European Habitats Directive and classified as Vulnerable by the International Union for Conservation of Nature. Next generation sequencing and geostatistical interpolation approaches were used to estimate intraspecific genetic diversity and generate continuous maps of genetic variation, based on both mitochondrial and nuclear data. Nuclear variation was assessed through single nucleotide polymorphisms (SNPs), identified by ddRAD sequencing of 212 *R. alpina* individuals sampled across Italy, while a fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was sequenced from the same specimens. Multiple diversity metrics were estimated for both datasets, including allelic richness, nucleotide diversity, heterozygosity, and haplotype richness. Local diversity values were calculated using a moving-window spatial approach, and continuous genetic diversity maps were generated through kriging interpolation. Because local diversity values for both SNPs and COI markers showed significant spatial autocorrelation, a restricted spatial permutation approach was applied to quantify the proportion of areas with the highest genetic diversity included within the Italian protected area system. Our results show that both nuclear and mitochondrial genetic diversity of *R. alpina* are generally well represented within the Italian network of protected areas, across most of the peninsula. However, populations from the Julian Prealps appear to be underrepresented within the current protected area system. In addition, patterns of mitochondrial diversity were not consistently correlated with nuclear genome diversity, highlighting the importance of integrating multiple genetic markers into conservation planning.

Ancient DNA insights into the historical distribution of the Galápagos pink land iguana (*Conolophus marthae*)

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Galápagos iguanas represent one of the most emblematic reptile radiations of oceanic islands. Among the three extant Galápagos land iguana species, the critically endangered pink iguana, *Conolophus marthae*, is currently restricted to a small area on the north-western slopes of Wolf Volcano (Isabela Island), whereas *C. subcristatus* is widespread across the archipelago. Recent population genomic analyses suggest that the *C. marthae* lineage may once have occupied a broader range across Isabela Island, consistent with its basal position within the *Conolophus* clade. Demographic reconstructions revealed opposite trajectories in the two species, with declining *C. marthae* populations and expanding syntopic *C. subcristatus*, suggesting a possible role of interspecific competition in the range contraction of the pink iguana. Additionally, stable isotope and dietary metagenomic analyses suggest substantial trophic overlap despite partial niche differentiation between the two species. Here, we present preliminary results from an ongoing ancient DNA study based on iguanas' subfossils recovered from lava tunnels across multiple islands of the Galápagos archipelago. Ancient DNA libraries were generated in dedicated clean-lab facilities, shallow-shotgun sequenced, and analyzed through competitive mapping against nuclear and mitochondrial reference genomes of both *C. marthae* and *C. subcristatus* to assess taxonomic affinities. Preliminary analyses revealed authentic ancient DNA signatures and, despite the generally low endogenous DNA content, one specimen recovered approximately 100 km south from the current distribution of the pink iguana shows stronger genomic affinity to the *C. marthae* lineage than to *C. subcristatus*. Although further analyses are ongoing, this result is consistent with the hypothesis that the pink iguana lineage historically occupied a substantially broader portion of Isabela Island before undergoing severe range contraction.

Genomic Insights into Population Structure and Historical Connectivity of the Antarctic Longfin Icedevil (*Aethotaxis mitopteryx*)

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Understanding population connectivity in the marine realm is fundamental for biodiversity conservation, fisheries management, and predicting species responses to environmental change. This is particularly relevant in Antarctic ecosystems, where extreme environmental conditions and unique evolutionary histories have shaped marine biodiversity. The longfin icedevil, *Aethotaxis mitopteryx*, is an elusive Antarctic notothenioid fish whose population structure and dispersal dynamics remains largely unexplored. To investigate connectivity patterns in this species, we used low-coverage whole-genome sequencing (LcWGS) on specimens collected from three geographically distinct regions: the eastern Weddell Sea, the eastern Antarctic Peninsula, and the Ross Sea, a region where the species had never been recorded before. Nuclear genomic variation and mitochondrial genome architecture were analyzed in parallel. Complete mitochondrial genomes were assembled to assess structural variation and infer population genetic patterns, which were then compared with nuclear SNP-based analyses. While mitochondrial data revealed no significant differentiation among populations, nuclear markers provided sufficient resolution to detect a clear genetic structure separating Ross Sea individuals from all other specimens. Inconsistencies between mitochondrial and nuclear patterns may reflect contrasting evolutionary timescales. Specifically, mitochondrial signatures could retain evidence of ancient gene flow linked to past oceanographic connectivity between the Ross and Weddell Seas, whereas nuclear genomes capture more recent divergence processes. Although previous hypotheses suggested extensive genetic homogeneity across the Southern Ocean, our results challenge this view by identifying a distinct genetic group in the Ross Sea. These findings provide novel insights into the connectivity of *A. mitopteryx* and support the hypothesis of historical water mass exchange between the Ross and Weddell Seas.

Genome-wide data disentangle range limits, introgression and phenotypic discordance in the Italian Aesculapian snake *Zamenis lineatus*

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Accurate range delimitation is central to conservation, but species boundaries may be difficult to define when phenotype, mitochondrial DNA and nuclear ancestry tell discordant stories. The Italian Aesculapian snake *Zamenis lineatus* is a narrow-range endemic of southern Italy and Sicily, historically confused with the widespread *Z. longissimus*. Previous integrative analyses revealed an unresolved pattern in Apulia: snakes from this region carry mitochondrial and low-resolution nuclear markers of *Z. longissimus*, yet show chimeric combinations of diagnostic phenotypic traits of both species. This raised two alternative hypotheses: Apulia may represent an extensive hybrid zone with widespread genomic admixture, or a region occupied by *Z. longissimus* populations retaining limited introgression and/or labile phenotypic traits. Here, we use genome-wide ddRAD data to test these alternatives and reassess the evolutionary and conservation significance of Apulian populations. Genome-wide SNP variation sharply separates *Z. lineatus* and *Z. longissimus*, while Apulian samples cluster with *Z. longissimus* and show little support for extensive genomic admixture. Thus, their intermediate phenotype of Apulian snakes is not mirrored by a broadly admixed genomic background. These results place most of Apulia outside the range of *Z. lineatus*, reinforcing its status as a geographically restricted endemic. At the same time, Apulian populations reveal a striking decoupling between genome-wide ancestry and diagnostic morphology. Their chimeric phenotype may reflect limited and asymmetric introgression of specific traits, persistence of ancestral phenotypic variation, or intrinsic lability of characters traditionally used for species identification. More broadly, this study illustrates how conservation genomics can disentangle range limits, hybridization and phenotypic uncertainty in contact zones, providing a robust framework for taxonomy, monitoring and conservation assessment.

Structural variants and their contribution to successful and unsuccessful colonizations in a marine gastropod

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In the current context of rapid environmental change and increasing anthropogenic impacts, understanding the evolutionary mechanisms underlying the uneven distribution of biodiversity is crucial for predicting species redistribution. While some populations struggle to survive or adapt, others persist or expand beyond their native ranges. Among the genetic factors potentially influencing these contrasting outcomes, structural variations (SVs) may play a key role in shaping population fitness. Here, we investigated the effects of SVs on colonization outcomes in the marine gastropod *Littorina saxatilis*, a species that provides a natural experiment of repeated colonization events, including both historical and recent, successful and failed range expansions. Using high-coverage (50×) whole-genomes from 149 individuals sampled across the species distribution range, we identified SVs from

POSTERS - Symposium 3: "Phylogeography and Conservation Genomics:
understanding evolutionary history to sustain species resilience"

paired-end short reads using a robust and widely adopted bioinformatic pipeline which includes a manual curation step. We then compared SV abundance and allele-frequency distribution in populations that underwent successful and unsuccessful colonization events to assess the potential role of chromosomal rearrangements in the colonization outcome. Finally, we used SVs to estimate genetic load quantifying deletions (DELs) in coding sequences, as potentially deleterious mutations that can affect gene expression and functioning. Overall, our results highlight the complex role of SVs during population range shift dynamics. These findings improve our understanding of the colonization process and the evolutionary dynamics underlying range shifts, shedding light on the genomic mechanisms distinguishing populations that persist from those that fail under rapid environmental change.

Genetic structure and demographic history of Sicilian and Calabrian loggerhead sea turtle nesting populations

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In marine environments increasing water temperatures due to global warming affect species distribution, and semi-enclosed marine basins such as the Mediterranean Sea show strong evidence of these phenomena. The loggerhead sea turtle, *Caretta caretta*, is a cosmopolitan marine vertebrate, and over the last decade its relative abundance within the Mediterranean has changed, with an increasing number of nests recorded across the Western basin. Conservation efforts are still needed, as it is still unclear how rapidly the southernmost latitudes will reach suboptimal temperatures for the species. Moreover, the impact of bycatch on *C. caretta* in the Mediterranean remains significant. Thanks to numerous projects carried out in recent years, monitoring of this species has increased, both at previously known sites and at newly identified ones. Sicily island hosts nesting events that were unstudied until a few years ago. It is still unclear whether this was due to insufficient sampling of a population that had long been present in the area, or to the recent appearance of nesting activity, plausibly as a combination of both factors. This study aims to genetically characterize the Sicilian population and to infer aspects of its demographic history in relation to the already known Calabrian population, to clarify how similar the two populations are today and whether they may also have been similar in the past. We address this topic using mitogenome sequences and low-coverage genomic data from the same whole-genome sequencing experiment, on a dataset of 78 and 86 samples collected from nests in Calabria and Sicily, respectively. Genetic diversity will be estimated by Φ_{ST} estimates and haplotype networks on mitochondrial sequences and by PCAngsd-based population structure analysis using genotypes likelihoods on nuclear data. Changes in effective population size through time will be inferred for both populations using site frequency spectrum-based approaches.

"Small" but certainly not insignificant: mitochondrial and nuclear markers uncover strikingly high genetic diversity in minnow (Phoxinus genus) Italian populations

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The only species of *Phoxinus* genus naturally found in Italy is *Phoxinus lumaireul*, which is currently considered at low risk of extinction. Given recent revisions of *Phoxinus* systematics, the plausible introduction of exotic *Phoxinus* species as live bait or forage fish in the past, as well as the declining size of Italian populations, this study aimed at i) genetically clarifying which *Phoxinus* species are currently present in Italy, and ii) describing both mitochondrial (mtDNA) and nuclear (nDNA) genetic diversity within the study area. Thus, 930 minnow individuals from 53 sites (mostly from Italy but including also reference sites in Europe) were characterized with the Cytochrome B marker, revealing an impressive 211 haplotypes (190 undescribed). Individuals were assigned to seven putative species according to species delimitation analysis: 661 samples from Adige, Danube, Esino, Isonzo, Livenza, and Po basins were identified as *P. lumaireul*, 225 from Adige, Danube, and Rhine as *P. csikii*, and the other samples from Danube, Rhine, and Ebro basins as either *P. marsillii*, *P. bigerri*, *P. septimaniae*, *Phoxinus* sp., or *P. phoxinus* clade 10. Bayesian clustering analysis on 12 microsatellites data suggested the occurrence of 14 clusters within the dataset; seven of these were associated to *P. lumaireul*, presenting a clear geographic structure. The genetic diversity observed at both mtDNA and nDNA was concordant with geographic distribution of individuals and populations, with a notable exception in the Adige Basin, which showed a gradient from sites with complete ancestry in a cluster associated to *P. lumaireul* to northern sites with complete ancestry in a cluster associated to *P. csikii*. The observed patterns of genetic diversity distribution were interpreted following different scenarios of natural and anthropogenic-driven gene flow between basins.

Evolutionary History of LRRK2 and PRKN in Leprosy and Parkinson's Disease

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LRRK2 (leucine-rich repeat kinase 2) and Parkin (encoded by PRKN) participate in shared molecular pathways and are implicated in the pathogenesis of Parkinson's disease (PD), leprosy, and other diseases. Whereas leprosy—one of the oldest and most stigmatizing human diseases—likely acted as a selective pressure during human evolution, PD, owing to its relatively late onset, is largely invisible to natural selection. Here, we investigated the evolutionary history of LRRK2 and PRKN across primates and human populations. We identified both genes as targets of positive selection in primates, but also detected strong constraints on LRRK2 disease-associated positions. In the case of PRKN, we report that gibbons carry a disease mutation (R33Q) that causes protein instability. Using ancient DNA, we reconstructed the temporal dynamics of LRRK2 and PRKN variants in European populations. The most common PD risk allele, LRRK2 G2019S—which also confers protection against salmonellosis—was absent in Paleolithic individuals but increased rapidly in frequency during the Early Neolithic. We propose that this rise was driven by selective pressure associated with the emergence of human-adapted *Salmonella* lineages. Analysis of leprosy susceptibility variants in LRRK2, PRKN, and other major loci showed that the frequencies of several risk alleles increased prior to the Middle Ages, when leprosy peaked in Europe. In contrast, we found little evidence for strong selection acting on these variants during the medieval leprosy epidemic itself. Together, our results provide a temporal framework for the emergence and possible positive selection of a major PD-associated mutation and suggest that selective forces—potentially unrelated to *Mycobacterium leprae*—shaped genetic susceptibility to leprosy before its medieval peak.

Population Genetics and Pathogen Identification of Double Inhumations from a Late Medieval Cemetery in Verona

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In recent years, ancient DNA (aDNA) analysis has become a powerful tool for investigating past human societies from various perspectives. The medieval cemetery discovered in Piazza Ardit, Verona, offers a unique opportunity to apply this tool to better understand social dynamics, biological and non-biological kinship, health status, and genetic diversity during a pivotal period of European history. The 13th and 14th centuries represent a time of significant political transition from communal structures to the Signorie, a shift that established Northern Italy as a primary centre of European trade and finance. Furthermore, the Black Death of 1348 was a catastrophic event with a profound impact on demography and social dynamics. The 31 selected individuals (part of the BIOCOMM project) belong to single and double inhumations from the 14th century and provide the opportunity to place the inhabitants of late medieval Verona within the broader context of European genetic diversity. Using aDNA to reconstruct biological kinship allowed us to explore relationships among individuals, identifying parent-offspring pairs as well as cousins and unrelated individuals. Moreover, the identification of pathogens has enabled the detection of peculiar pathogens such as *Actinobaculum madurae*, but it could not confirm the presence of *Yersinia pestis*, probably due to the rarity of the septicemic form of plague, the only case in which the aetiological agent enters the bloodstream. However, a promising individual will be subject to capture sequencing to detect *Yersinia pestis*. The evolution of the human species extends beyond genetics; it is deeply intertwined with complex social dynamics and global crises, which can only be fully understood through multidisciplinary approaches integrating biological anthropology, archaeology, and history.

Genomic and bioarchaeological evidence for a multi-stage mortuary landscape in the Late Upper Paleolithic Arene Candide Cave (Italy)

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The Epigravettian “necropolis” of Arene Candide Cave, located in Northern Italy and dated between ca. 12,800–11,800 cal BP, represents an exceptional context for reconstructing the biological composition, social structure, and funerary practices of Late Pleistocene hunter-gatherers. Archaeo-anthropological analyses revealed burial practices and ritual behaviors intended to establish symbolic connections among individuals, potentially reflecting kinship relationships or shared congenital conditions. Among the 17 analyzed samples, 13 individuals were genetically characterized to reconstruct their biological profiles, genetic heritage, and familial relationships. Shotgun sequencing and target enrichment approach were used to recover mitochondrial and nuclear genomes. Preliminary results identified five females and eight males, while kinship analysis revealed close biological relationships among several individuals, confirming the archaeological interpretation of the depositional context of the burials. Genome-wide analysis of runs of homozygosity (ROH) shows that all individuals exhibit a substantial excess of long ROH segments, consistent with close parental relatedness. Additionally, all mitochondrial DNA haplogroups are closely related with several individuals sharing identical haplotypes, suggesting a common maternal lineage and a small population size. Given that several individuals present congenital diseases, ongoing genomic analyses aim to investigate the genetic basis of the observed disorders.

Diachronic paleogenomic study of Central Italy: analysis of the remains from the Palazzone necropolis and the Hypogeum of the Volumni (6th century BCE–1st century CE)

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This project aims to reconstruct the genetic history of central Italian populations through the analysis of human remains from Etruscan-Umbrian archaeological contexts dating between the 6th century BCE and the 1st century CE. The genomic analyses were carried out on 60 individuals recovered from the Palazzone necropolis and other funerary contexts in the Perugia area associated with Etruscan and Umbrian cultures. These complexes are located on opposite banks of the Tiber River, providing an ideal framework for exploring genetic differences between geographically close but culturally distinct communities. While the Etruscans of Tuscany have been extensively investigated from a genetic perspective, Umbria remains largely unexplored. We initially assessed endogenous DNA preservation through shallow shotgun sequencing. The analyzed samples showed variable, yet suitable, endogenous DNA preservation. Preliminary allele-frequency analyses enabled the investigation of genetic variability through Principal Component Analysis (PCA), revealing that the individuals cluster into two distinct groups, both falling within the genetic variability of Central Italy. Kinship analyses performed using READ, KIN and TKGWV2 provided preliminary evidence of close biological relationships among individuals. Future analyses will focus on identifying genetic changes over time potentially associated with migrations, historical events, and cultural exchanges over time, as well as evaluating patterns of genetic admixture between Umbrian, Etruscan, and neighboring populations.

Traces of the invisible: an integrated sedaDNA analytical framework for Italian archaeological sites

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Ancient DNA recovered from archaeological sediments (sedaDNA) is reshaping paleoanthropology by revealing species invisible to macro and micro remain studies, thus improving environmental and archaeological interpretations. Although highly fragmented and affected by inhibitors, sedaDNA can survive for tens of thousands of years and be recovered by dedicated extraction and NGS protocols. Target enrichment using DNA/RNA probes increases the proportion of informative molecules and prevents microbial DNA from overwhelming sequencing output. Despite requiring a priori selection of taxa, it remains the most effective strategy for retrieving meaningful genetic signals from sediments. To date, sedaDNA studies for about 20 archaeological contexts up to 250,000 years are available and recent findings show that sedaDNA can persist even in warm regions, indicating strong potential for Mediterranean contexts. Italy, however, remains largely unexplored, and existing probe panels are poorly suited to its past environmental diversity. We are developing an optimized sedaDNA analytical framework for the Italian archaeological record through a multidisciplinary collaboration spanning molecular anthropology, archaeology, archaeozoology and paleobotany. We developed new probe panels targeting faunal, plant, fungal and lichen taxa. Markers useful for species- or genus-level identification were selected for a curated list of 882 species of anthropological interest, and the resulting probes are being tested on sediment samples from different prehistoric and protohistoric Italian sites. By implementing cutting edge sedaDNA methodologies, our project will provide a workflow applicable to Italian contexts

improving the reconstructions of ancient environments and of human subsistence strategies beyond the limits of traditional archaeological evidence.

The impact of transposable elements in the genomic plasticity of the Asian tiger mosquito *Aedes albopictus*

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Structural variation forms the basis of genome plasticity, and there is growing evidence that it can also contribute to adaptive evolution. Transposable elements (TEs), which can replicate and insert into new genomic loci, are a key contributor to this plasticity. Our aim was to assess whether TE mobilisation increases under thermal stress and whether it represents a consequent genetic substrate for adaptation. For this analysis, we focused on the Asian tiger mosquito (*Aedes albopictus*), an invasive species with remarkable environmental tolerance and a TE-rich genome (~55%). In the laboratory, we reared Foshan strain larvae under heat stress and compared their fitness and TE landscape with those of a control population reared at 28 °C. Lab-reared, heat-stressed mosquitoes exhibited reduced fitness compared to the control group. Preliminary genome-level analyses suggest a higher number of novel TE insertions in heat-stressed families, indicating temperature-dependent TE mobilisation. This could eventually contribute to new beneficial mutations and rapid adaptation. We applied the same TE insertion detection approach to six different natural *Ae. albopictus* populations, three of which were sampled in northern Italy, where winters are cold and summers are mild, and three in southern Italy, where winters are warm and summers are hot. Further analysis of these populations will clarify the evolutionary role of TEs in *Ae. albopictus* and reveal the characteristics of the TE landscape of Italian mosquitoes.

Tracking diatom genomes in nature reveals ecological diversification across coastal environmental gradients

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Diatoms are highly diverse microbial eukaryotes with major ecological roles in marine ecosystems. A growing number of genomes from cultured isolates is now available, providing access to the genetic basis of diatom physiology, life cycles and ecological strategies. Yet, a central challenge remains: linking these genomic resources to the occurrence and activity of closely related lineages in natural, fluctuating environments. We address this question using NEREA, a coastal multi-omics observatory in the Gulf of Naples embedded within the long-term ecological framework of LTER-MC. We combine genome-resolved metagenomics, metatranscriptomics, metabarcoding and environmental metadata to track diatom genomes in situ across monthly samples, different depths and size fractions. Rather than treating community omics only as a biodiversity survey, we use read recruitment as a bridge between isolate-based genomic resources and ecological dynamics in nature. We focus on dominant and ecologically relevant genera, including *Chaetoceros* and *Pseudo-nitzschia*. *Chaetoceros* is a major component of the coastal diatom community, and multiple reference genomes allow us to compare species-specific genomic repertoires and transcriptional activity across environmental regimes. *Pseudo-nitzschia* represents an established system for studying cryptic diversity, species-complex dynamics, bloom recurrence and population structure in the Gulf of Naples. Previous work showed that *P. multistriata* populations display temporal dynamics shaped by clonal expansion, recombination and mating-type variation; NEREA now places these processes within a broader functional context. By following diatom genomes directly in environmental meta-omics data, our work connects organismal genomics with natural community dynamics and provides a

POSTERS - Symposium 5: “The evolution of ecological diversification: mechanisms, interactions, and outcomes”

framework to investigate how microbial eukaryotes persist, partition ecological space and express alternative functional strategies in changing coastal environments.

Autopolyploidization presents a transient and potential-rich window of increased transcriptional plasticity in *Arabidopsis arenosa*.

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Whole-genome duplication (WGD, polyploidization) is a pervasive feature of Eukaryote evolution and often viewed as a source of evolutionary success and novelty, meaning a macromutation leading to higher fitness (i.e. “hopeful monsters”). Yet, the mechanisms behind the (occasional) success of nascent polyploids remain still elusive, especially from a transcriptomic point of view. Theory suggests that duplicated genetic networks are characterised by enhanced redundancy and higher output variation, promoting the exploration of the adaptive landscape during stressful times. Artificially synthesized neo-polyploids provide an exciting system to test this, however, empirical studies comparing co-expression network patterns between natural and synthetic ploidies of the same species in an evolutionary context are lacking. We compared diploid, synthetic and naturally established autotetraploid populations of *Arabidopsis arenosa* to investigate short- versus long-term effects of polyploidy on gene expression complexity and plasticity under water deficiency stress. Transcriptomic profiling revealed that synthetic neo-tetraploids explored the broadest expression space and exhibited the highest number of stress-responsive genes. Co-expression network analyses demonstrated that the network of neo-tetraploids was fragmented into multiple highly connected modules, with stress-responsive genes preferentially acting as inter-modular “bridges.” In contrast, diploids and established tetraploids exhibited lower expression variation, more modular architectures, with stress response genes embedded within well-defined modules. Together, our results indicate that WGD induces a transient phase of transcriptomic expansion and network disorganization that broadens the phenotypic landscape, followed by evolutionary stabilization and finally retention of some advantageous novelties. This supports the view of neo-polyploids as “hopeful monsters”.

Evolutionary genome dynamics in malaria vector *Anopheles coluzzii* mosquitoes over the early phases of laboratory colonization

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Laboratory-reared organisms are assumed to be accurate representations of their wild counterparts. However, the process of establishing a colony from a small number of founders under conditions that significantly differ from the natural environment may dramatically influence genetic composition of the colonized population. As a result, various aspects of physiology, fitness, and behavior of the resulting individuals may also be affected. Here we investigated genomic changes occurring during laboratory adaptation in the malaria vector *Anopheles coluzzii*. The newly colonized progenies of indoor-resting blood-fed females collected from a field population in Mali were sampled across multiple generations and allele frequency changes were tracked through whole-genome sequencing of pools of 50 females. Our analysis revealed a marked decline in genetic diversity, most likely driven by a combined effect of small effective population size and strong selection. We observed dramatic allele shifts at multiple loci, occurring primarily in the earliest generations post-colonization. Among the most affected were (i) the voltage-gated sodium channel gene, with alleles conferring knock-down resistance (kdr) to pyrethroids and DDT highly advantageous in the field under insecticide pressure, but associated with high fitness costs in the absence of insecticides, (ii) paracentric inversions on chromosome 2, in particular inversion 2La, and (iii) a cluster of mosquito immunity-related thioester-containing protein (TEP) genes on chromosomal arm 3L. Collectively, these results illustrate how stochastic processes and selective pressures rapidly reshape the genomic architecture of *A. coluzzii* during early laboratory colonization. Understanding these dynamics is essential not only to elucidating general patterns of population evolutionary trajectories, but also for accurately interpreting the ecological relevance of experimental outcomes in various mosquito studies.

Uncovering Adaptive Traits in Legumes through Novel Genotyping Approaches

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Ecological diversification is shaped by complex interactions between a species' genome and its environment, determining adaptive outcomes across landscapes. Legumes are essential for ecological sustainability and food security, yet decoding the genetic basis of their environmental adaptation is often obstructed by massive and highly repetitive genomes. To resolve the mechanisms underlying this diversification, the GENUINE (Genomic mEthods to ideNtify fUture cllmate resilient geNotypes in lEgumes) project presents a novel, cost-efficient genotyping platform based on a Cas9-mediated repeat depletion system. This pipeline selectively removes repetitive elements to enrich functional, single-copy genomic sequences. We deployed this strategy across genetically diverse germplasm panels of lentil (*Lens culinaris*), pea (*Pisum sativum*), and faba bean (*Vicia faba*), three economically vital crops representing contrasting agro-environmental regions. Preliminary analyses in lentil and pea demonstrate that Cas9-driven depletion effectively focuses sequencing efforts on informative regions, maximizing genotyping efficiency. Merging these genomic profiles with spatial environmental variables within a landscape genomics framework allows us to dissect the genotype-environment interactions governing adaptation. Ongoing and future genome-wide association studies (GWAS) will further connect these adaptive loci with critical agronomic traits. Ultimately, this research provides a clearer view of the interactions and mechanisms driving ecological diversification, yielding vital outcomes in the form of innovative genomic tools to support climate-resilient agriculture under future global warming scenarios.

MicroRNA Control of Mitochondrial Stress Responses: A Conserved Mechanism Linking Cellular Physiology to Ecological Diversification

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Mitochondria are central to adaptive responses to environmental variability, integrating energy metabolism, redox balance, and stress signalling. In aquatic environments, where oxygen and nutrient availability fluctuate rapidly, mitochondrial regulation represents a key interface linking environmental stress to evolutionary diversification. MicroRNAs (miRNAs) provide rapid, coordinated post-transcriptional control of gene networks underlying such plasticity. Here, we propose a comparative framework to investigate the role of conserved miR-17~92 cluster in mitochondrial stress adaptation across vertebrates. Using the teleost *Takifugu rubripes* as a model, miRNA expression dynamics will be quantified under stress conditions, with validation in *Oryzias latipes* and comparative analyses in human cell lines. Our preliminary data, indicates that chemical hypoxia and oxidative stress significantly reduced cell viability in a dose-dependent manner and induced coordinated downregulation of miR-17~92 family expression, revealing stress-responsive modulation across the cluster. Moreover, concomitant inhibition of miR-17, miR-20 and miR-92 in two different human cell lines allowed to identify, by Gene Set Enrichment Analysis (GSEA) of transcriptomic data, the modulation of cellular respiration, oxidative phosphorylation, mitochondrial gene expression and mitochondrial translation in both cell lines. Functional consequences will be assessed with quantitative measurements of mitochondrial activity, reactive oxygen species production, and transcriptomic profiling in fish cell lines to identify conserved and lineage-specific targets. Extending this framework to teleost models which occupy distinct ecological niches will enable comparative investigation of how miRNA-mediated regulation of mitochondrial stress responses contributes to lineage-specific ecological diversification, providing a mechanistic link between cellular physiology and evolutionary outcomes.

Effects of fragmented landscapes on the evolution of thermal tolerance in soil arthropods.

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Species' tolerance to environmental conditions relies on a delicate balance between phenotype, genotype, and environment. It varies across space and is shaped by evolutionary forces such as natural selection or genetic drift: discerning the footprint of these processes is challenging, and they are greatly impacted by the species' own dispersal abilities and the levels of habitat fragmentation. Collembola (springtails), soil-dwelling arthropods with limited dispersal ability, represent an ideal system in which to investigate the process of adaptation to the environment in a stationary taxon and distinguish the role of selection versus drift. Previous studies found small differences in heat tolerance between populations but strong correlation with latitude across a large geographical range in a collembola species, suggesting adaptation to the local environment. In this study, we evaluated temperature tolerance in 23 populations of *Entomobrya nicoleti* sampled across Denmark, and detected substantial differences in heat and cold tolerance across populations over a much smaller geographical range. To understand what has shaped these phenotypic differences, we integrated whole-genome data to environmental metadata and conducted genotype-phenotype-environment association analyses to identify loci potentially linked to heat and cold tolerance, and whether environmental variables are associated to a specific phenotype. We additionally distinguished a set of candidate genes previously identified as involved in temperature tolerance in arthropods. By combining genome-wide data, phenotype analyses, and environmental parameters, we got closer to understanding the potential and the drivers of adaptation to the environment and highlighted the consequences of increasing habitat fragmentation on vulnerable non-dispersive species.

Genomics of past and future climate adaptation in sessile oak (*Quercus petraea*)

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Many forest tree species, such as European white oaks (*Quercus* section *Quercus*), inhabit wide geographical ranges where fitness optima and the underlying genotypes vary along environmental gradients. These signatures of adaptation can be studied using landscape genomics. However, climate change might disrupt these associations because of the slow pace of tree evolutionary response to new climatic regimes. Here, we analyse whole genomes of 791 sessile oak (*Q. petraea*) individuals from 72 populations (13 natural populations and 59 provenances grown in four common garden experiments established in the 1980s) from and from Italy to Norway and from Scotland to Georgia. Additionally, we use whole-genome data of 116 individuals from populations of other, hybridising white oak species for species assignment and hybrid identification. This large dataset, along with monthly climatic data from CHELSAcruts, allows us to obtain a picture of the species' adaptive genomic patterns in relation to climate and to predict its evolutionary fate at the continental scale under future climate change. We combine different genotype-environment association approaches to investigate range-wide genomic signatures of climate adaptation. We use genomic offset metrics to describe the genomic change

needed by a population to track the local climate under climate change scenarios for 2050 and 2100 and to assess maladaptation risk at each site. We support our findings by investigating whether genomic offset values predict tree decline in natural stands and by studying climate sensitivity derived from dendrochronological time series of a subset of trees. Our study will predict which oak populations face a greater risk of declining in the near future and inform forest management by identifying as reservoirs for assisted gene flow those provenances that carry alleles deemed to be beneficial under the changing climate.

The diversity of transposable elements landscapes in Culicomorpha

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The infraorder Culicomorpha comprises nematoceran insects like biting midges and mosquitoes. This taxon exhibits high variability in genome size and transposable element (TE) abundance, particularly within the Culicidae family (Anophelinae and Culicinae mosquito subfamilies). To investigate the dynamics driving their evolution, we performed a comparative evaluation of TE content and diversity across 59 Culicomorpha species, focusing notably on Anophelinae (n = 34) and Culicinae (n = 15). Raw sequencing data derived from single individuals and obtained from public databases were used to (re)assemble their nuclear genome, estimate genome size, and quantify the TE content. A time-calibrated phylogeny based on BUSCO genes was also inferred to characterize the mode and tempo of TE dynamics across the evolutionary history of these species. We find genome size and TE content to be strongly correlated: Culicinae genera *Aedes* and *Culex* have the largest genomes and TE loads, while Anophelinae genomes are generally small and TE-depleted. Applying Shannon's and Simpson's ecological indices to TE order abundances revealed a strong positive correlation between genome size and TE diversity in Culicinae only, indicating that genome expansion in Culicomorpha is determined by the combined proliferation of multiple TE orders, rather than a predominant one. We also found no significant phylogenetic correlation between genome size or TE content and the ratios of non-synonymous to synonymous substitutions or of radical over conservative substitutions, suggesting that the large Culicinae genomes did not emerge as a result of relaxed selection. Finally, a saltational model best fits genome size and TE content evolution, with a jump event at the base of Culicinae, pointing to punctual rather than gradual processes underlying genome size change in this clade.

Genetic basis of resistance in Norway Spruce (*Picea abies*) to the European Spruce Bark Beetle (*Ips typographus*)

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The frequency and severity of outbreaks of the European spruce bark beetle (*Ips typographus*) have increased markedly under ongoing climate change. Rising temperatures and drought stress weaken Norway spruce (*Picea abies*) defenses and facilitate large-scale bark beetle infestations, resulting in considerable ecological and economic damage across European forests. However, mortality is rarely 100%, indicating that there may be genetic variation associated with spruce defense mechanisms. Identifying the genetic factors underlying resistance to bark beetle attack may improve our understanding of adaptive variation in spruce populations and contribute to the development of more resilient forests via improved management practices. We established long-term monitoring plots in proximity to active bark beetle outbreaks in two national parks, Šumava National Park in Czechia and Berchtesgaden National Park in Germany. By the end of the monitoring period, five plots in Šumava had become infested, comprising 279 trees and exhibiting a relatively high mortality rate of 75.6%. In contrast, mortality in Berchtesgaden was only 20% among 359 trees distributed across five plots. Cambial tissue was collected for DNA extraction. To address the large genome size of Norway spruce, we employed genotyping-by-sequencing (GBS), resulting in ~2M markers with reads in over 90%

POSTERS - Symposium 5: “The evolution of ecological diversification: mechanisms, interactions, and outcomes”

of annotated genes. A survival model was developed to remove variance associated with environmental and other confounding effects from the survival signal. The genetic architecture of spruce survival was investigated using the corrected survival phenotype in a genome-wide association (GWAS) model.

From genome to populations: reference assembly and population genomics of the Galápagos endemic *Darwiniothamnus* genus

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How is genetic diversity and population structure distributed within a young, multi-species island radiation? *Darwiniothamnus* is endemic to the Galápagos, where it radiated into 3 species. Here, we generate a de novo genome assembly, with gene and transposable element annotation, for *D. tenuifolius*, revealing a likely tetraploid genome that we resolve into parental subgenomes, and use this assembly as reference for high coverage (30X) short-read resequencing of 56 individuals across all species. Using this dataset, we characterize population structure and genetic diversity within and between species - across Isabela and Santa Cruz islands - and assess pairwise relatedness within and between populations. This work will provide the first genome-wide characterization of population structure and genetic diversity across the *Darwiniothamnus* radiation.

Evolutionary insights into the recent Mediterranean expansion of *Pinna rudis*

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The recent increase of *Pinna rudis* populations in the Mediterranean Sea, following the dramatic decline of its congener *Pinna nobilis*, raises important questions about the evolutionary and demographic processes shaping the species across its distribution range. To investigate these patterns, we analysed 115 mitochondrial DNA sequences based on a concatenated COI–16S fragment obtained from Atlantic and Mediterranean populations, applying phylogenetic and phylogeographic methods. Our results suggest that the common Atlanto–Mediterranean ancestor of *P. rudis* dates back to approximately 1.3 million years ago, whereas the mitochondrial diversity currently observed within the two basins likely originated during the mid-Pleistocene. This timeframe coincides with repeated climatic oscillations and marked sea-level changes that may have promoted episodes of isolation and reconnection among populations. Overall, our analyses based on the mitochondrial perspective indicate an early sign of genetic structuring across basins. In line with this pattern, demographic analyses revealed contrasting historical trajectories, with Atlantic populations exhibiting signals of expansion, while Mediterranean populations showed more complex demographic dynamics. In addition, two instances of natural hybridisation with *P. nobilis* were detected in the central western Mediterranean. Taken together, these findings highlight the role of Pleistocene environmental

instability in shaping the mitochondrial evolutionary history of *P. rudis* and provide new insights into the processes underlying its current expansion within Mediterranean ecosystems. These patterns should be further corroborated in the future by nuclear analyses.

An integrative approach for species delimitation in the highly diversified stingless bee genus *Trigona* (Apoidea: Meliponini)

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Applying DNA-barcoding for species identification to Neotropical stingless bees is challenging, but its usefulness has not yet been fully evaluated. We examined the efficiency of *COI*-barcoding to identify species within the genus *Trigona*, currently subdivided into 9 species-groups. To do so, we considered 99 newly collected (and morphologically identified) specimens across 9 Peruvian Departments and 1286 *COI* *Trigona* sequences retrieved from BOLD. To examine species partitioning, two distance-based (ABGD, ASAP) and one tree-based (mPTP) species delimitation methods were applied on the whole dataset. We also performed an ecological niche analysis to support distinction among some recognized allied species. *COI*-barcoding was proven to be reliable for 7 (out of 21) species of *Trigona*, also well-distinguished morphologically. In all other instances, we detected a morphological vs. molecular ID mismatch, mostly affecting sibling species (e.g. *T. chanchamayoensis* vs. *T. muzoensis*). Species delimitation methods were also somewhat incongruent, with ABGD and ASAP oversplitting some clusters/species. This was particularly evident within the '*fulviventris*' group of species, where the actual distinction between *T. fulviventris* and *T. guianae* is blurred by slight overlapping of some morphological traits and distribution. However, the environmental clustering analysis also showed a lack of ecological distinction between these two species. All the above mentioned issues could be related to the absence of a reliable barcoding gap due to recent speciation processes, as well as for the incompleteness of the existing dichotomous morphological keys/databases. A "reverse taxonomy" and quantitative approaches (e.g. morphometry, chemical and genomic analyses) combined with the optimization of the existing tools for taxonomic identification of Meliponini are needed to resolve

such ambiguities and properly conserve the local biodiversity of these key pollinators of the tropical forests.

Probing ecdysone biosynthesis in arthropods: a functional analysis of Halloween genes in the mite *Varroa destructor*

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Ecdysone plays a central role in the regulation of moulting and other key aspects of development and reproduction in arthropods. This hormone is synthesized in specialized endocrine glands and tissues that vary across major lineages. Its biosynthesis involves a series of enzymes encoded by so-called Halloween genes, which mediate the conversion of dietary cholesterol into the active hormone. In recent years, these genes have increasingly attracted interest in applied biology as potential targets for RNA interference-based strategies, aimed at reducing infestations by arthropod parasites. However, despite the conservation of ecdysone across lineages, the pathway leading to its biosynthesis has extensively diversified throughout arthropods evolution, a phenomenon called ‘developmental system drift’, the change in the morphogenetic or gene-regulatory underpinnings of a conserved trait. To date, a comprehensive comparative functional analysis of this pathway across the whole taxon is still lacking, hampering the exportability of RNA interference methods across species. Here, we present some preliminary results on the phenotypic effects of silencing selected Halloween genes in the mite *Varroa destructor*, a major parasite of the honey bee. Our findings provide initial insights into conservation and divergence of ecdysteroidogenesis in Arthropoda. This study in applied evolutionary biology contributes to our understanding of the evolution of a key hormonal pathway in arthropods and probes its potential for the development of innovative biotechnological tools for parasite control.

The Reconstruction of Enrica Calabresi's biography and scientific contributions: A Passionate Scientist, Zoologist and Teacher in the Early 20th Century

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This research project aims to reconstruct the biography and scientific profile of Enrica Calabresi by examining her scholarly contributions within their socio-historical context. It investigates the personal and academic challenges she faced, with particular attention to the ways in which Jewish identity and gender shaped her professional career. A central objective of the project is the comprehensive recovery of Calabresi's scientific publications in order to ensure access to her work. The research also focuses on the zoological specimens she studied, especially those held in the entomological and herpetological collections of the “La Specola” Museum of Natural History in Florence, where Calabresi worked from 1914 to 1933. The study has already located the entomological type material deposited in the collections of the following museums: “La Specola”, Museum of Natural History “Giacomo Doria” in Genoa, Staatliches Museum für Tierkunde in Dresden, Muséum National d'Histoire Naturelle in Paris, and Institut Royal des Sciences Naturelles de Belgique in Brussels. The specimens held at “La Specola” have been photographed together with their original labels and systematically catalogued in tables recording the main collection data. The same methodology is now being extended to the identification and digitisation of Calabresi's herpetological type material, with the aim of providing a more accurate understanding of her zoological expertise. The project also reconstructs the collection sites of these specimens to highlight the zoogeographical dimension of her work. By digitising all her type material, the research seeks to facilitate scholarly access and to enhance the visibility of her pioneering contributions to Entomology and Herpetology. Finally, the project includes a rigorous archival investigation of documents held in the archives of the University of Florence, the University of Pisa, and the secondary schools where Calabresi worked.

Venomous or not? The first transcriptomic profile of a nutmeg snail (Neogastropoda, Cancellariidae)

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The order Neogastropoda is the most diversified group of marine molluscs. Most gastropods are carnivorous and have evolved a unique array of physiological and biochemical adaptations to deal with their preys, predators and hosts. These include production and delivery of acidic or neurotoxins, asphyxiation, and hematophagy. However, in most lineages the trophic adaptations are still uncharacterized at the biochemical level. The family Cancellariidae, including ~300 described species known as nutmeg snails, is the earliest diverging clade of Neogastropoda, thus representing a key target to investigate early diversification and reconstruct the ancestral trophic adaptations of this lineage. However, Cancellariidae are seldom found alive, leading to a poor understanding of their biology and ecology. Anecdotal field observations on a few species have reported associations with fishes or other gastropod, with ingestion of organic fluids, blood, or skin fragments. Here, we describe the first tissue-specific transcriptomic profiling of *Scalptia contabulata* (G. B. Sowerby I, 1832), a cancellariid species often found exploiting large gastropods as food, by scraping their skin. An array of tissues from multiple specimens, including foot, osphradium, salivary glands, and accessory salivary glands, were excised and processed for transcriptome sequencing. A differential expression analysis was performed to identify and annotate the overexpressed transcripts for each tissue, and those of tissues directly involved in feeding were further investigated to characterize the bioactive secretions of *S. contabulata* and compare them with available data on other neogastropod species. Our high-quality transcriptome (99.4% BUSCO) showed distinct profiles among tissues, clearly separating secretory and non-secretory tissues. The characterisation of the salivary secretion shed new light on the trophic ecology of this species and of cancellariids feeding adaptations.

Less is better! A new cluster analysis to reduce SNP sampling in the Japanese beetle

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Understanding the phylogeographic history of invasive species is crucial to trace their route of introduction and dispersal. *Popillia japonica* is a major agricultural pest that has spread from its native range in Japan to North America and Europe, causing extensive damage to crops and ecosystems. Recent studies explored the genomic landscape of this invasion by employing large-scale SNP datasets obtained through WGS and remapping to a reference genome. This comprehensive dataset has been analysed to successfully trace the beetle's invasion routes from Japan to the United States, the Azores, and Switzerland. In this study, we aimed at improving the SNP selection process by drastically reducing the number of markers while retaining the resolution necessary to reconstruct key phylogeographic patterns. We implemented two complementary approaches: (1) a custom random SNP selection method (rAIMdom), and (2) a targeted selection of ancient informative markers based on minimum allele frequency thresholds (AIMforce). By downscaling the number of SNPs to 10x and 100x, we evaluated the ability of reduced marker sets to recapitulate previously established invasion pathways and population dynamics. Our results indicate that even with a significant reduction in the number of SNP employed, both selection strategies successfully produced datasets that can capture the core phylogeographic signal, demonstrating the robustness of the invasion patterns across distinct geographic regions. By minimizing the number of required SNPs, our methods lower sequencing costs, simplifies downstream analyses, enhances the feasibility of large-scale monitoring programs and opens to the possibility to design cost-accessible genotyping chips.

Integrating Secondary Structure Information Enhances Phylogenetic Signal in Mitochondrial Protein Coding Genes

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Accurate phylogenetic inference requires models accounting for heterogeneity in molecular evolution. Mitochondrial protein-coding genes exhibit considerable compositional and functional variation across structural regions, often overlooked in standard partitioning strategies. We introduce TRAMPO (TRAnsMembrane Protein Order), a pipeline incorporating predicted secondary structural features (matrix-facing, transmembrane, and intermembrane-facing domains) into phylogenetic partitioning schemes. Applied to seven mitochondrial datasets spanning crustaceans, hexapods, and vertebrates, we evaluated eight partitioning strategies combining codon position, strand, and secondary structure. Transmembrane helices showed thymine enrichment at second codon positions and hydrophobic amino-acid composition, reflecting domain-specific constraints. Maximum likelihood analyses under Markov models of varying complexity, combined with multiple rate-heterogeneity models, examined their interaction with partitioning strategies. Structural partitioning consistently improved model fit and reduced heterogeneity, reflected in lower AIC values and more homogeneous partitions, while reducing computational time. Second codon positions encoding transmembrane helices were retained as distinct partitions even in mammals and vertebrates, underscoring their conserved evolutionary signal. Quartet-distance surveys confirmed structurally informed models produced more consistent topologies. Benefits were most pronounced at intermediate evolutionary depths and declined in ancient clades, where substitutional saturation accumulates. Lowest-AIC models did not always yield the most congruent topologies, highlighting limitations of information criteria. Secondary structural features harbour meaningful phylogenetic signal; incorporating them improves tree reconstruction and mitigates heterogeneity. TRAMPO is a scalable, open-source tool for mitochondrial phylogenetics.

Wastewater surveillance as an early warning system for SARS-CoV-2 outbreaks

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Wastewater-based epidemiology is a valuable tool for monitoring SARS-CoV-2 circulation, as viral shedding through the gastrointestinal tract also occurs in asymptomatic and presymptomatic individuals. Consequently, wastewater surveillance represents an effective approach for detecting emerging variants before they become widespread and symptomatic within the population. In this context, the study aimed to conduct comparative genomic and phylodynamic analyses of SARS-CoV-2 Omicron sequences obtained from wastewater and clinical samples to evaluate whether wastewater-based genomic surveillance can capture the viral diversity and evolutionary dynamics observed in the human population. Pune, India, was selected as the study setting because publicly available genomic repositories provided temporally comparable wastewater and clinical SARS-CoV-2 genomic datasets from the same geographical area. In addition, a larger clinical genomic dataset spanning different phases of Omicron circulation was analysed to reconstruct the broader evolutionary dynamics of the variant. Clustering analysis showed no clear genetic structuring associated with sample type, indicating substantial overlap between wastewater and clinical sequences. Bayesian Skyline Plot analysis identified two major increases in viral population size shared across both datasets. Notably, increases detected in wastewater samples occurred approximately 1.5 months earlier than in clinical samples, further supporting the role of wastewater surveillance as an effective early warning system for SARS-CoV-2 outbreaks. The present study is based on a retrospective analysis, and the results clearly support the predictive potential of

wastewater surveillance for anticipating outbreaks subsequently recorded in the human population. Accordingly, continuous monitoring of wastewater could represent a powerful tool for the early detection of emerging SARS-CoV-2 variants and for strengthening preparedness against future outbreaks.

Evolution of protein effectors in *Rickettsiaceae* (*Alphaproteobacteria*) and their role in host interactions

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The *Rickettsiaceae* family comprises a diverse group of obligate intracellular bacteria, including both pathogens transmitted by hematophagous arthropods (e.g., *Rickettsia* and *Orientia*) and non-pathogenic taxa, such as the insect-associated *Tisiphia*, the ciliate-associated *Megaera* and *Trichorickettsia*. In this study, we sequenced and assembled the genomes of two *Tisiphia* strains from a mite and a tick, using short and long read sequencing technologies. These two novel genomes were integrated into a representative dataset comprising over 100 *Rickettsiaceae* genomes from seven different genera, hosted by diverse eukaryotic organisms. Our analysis identified the newly sequenced isolates as representing two novel species within the *Tisiphia* genus. We focused on the homologs of known proteins involved in host interactions in pathogenic *Rickettsiaceae*, particularly Surface cell antigens (Sca) and RickA proteins. In *Rickettsia*, these proteins are involved in host cell entry and in modulation of host actin for bacterial motility, respectively. Our findings indicate that RickA is restricted to *Rickettsiaceae* lineages associated with multicellular hosts. In contrast, the Sca protein family is more widespread across *Rickettsiaceae*, frequently appearing in multiple copies per genome, with significant variation in copy number even among closely related strains. Notably, phylogenetic analyses revealed that most Sca sequences exhibit patterns that are not consistent with the species phylogeny, suggesting a complex evolutionary history. Conversely Sca proteins of *Rickettsiaceae* from terrestrial hosts clustered separately from those originating from aquatic environments. Finally, in silico structural predictions using AlphaFold for selected Sca proteins suggested distinct protein–protein interaction profiles among different Sca paralogs, consistent with potential differential roles in host specificity and virulence.

Core-Gene Consensus Delimitation (CGCD): A universal genome-wide framework for microbial species delimitation

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Defining species from genomic data is still a major challenge, especially when genomes are highly plastic and evolutionary boundaries are blurred. Many existing species-delimitation methods rely on single genes, fixed thresholds, or sequence-identity cutoffs that mask gene-level signals, limiting their applicability to genome-scale data. Here, we introduce Core-Gene Consensus Delimitation (CGCD), a novel, gene-based and threshold-free framework for species delimitation that extends distance-based methods to the core-genome level. CGCD integrates conspecificity matrices derived from individual genes and combines them into a consensus signal across hundreds to thousands of loci. This enables the application of traditionally single-locus algorithms — Automatic Barcode Gap Discovery (ABGD) and Assemble Species by Automatic Partitioning (ASAP) — within a multi-locus genomic context. We demonstrate the performance of CGCD using *Acinetobacter baumannii* as a case study, a taxon known for extreme genomic plasticity and complex population structure. Analysis of 47 genomes from the VUB collection revealed 11 highly stable groups, consistently recovered across a wide range of gene-level co-occurrence thresholds using both ABGD and ASAP. Large-scale validation with 856 additional genomes confirmed the persistence and robustness of these groups. Phylogenomic and accessory-genome analyses showed that CGCD-defined groups correspond to distinct evolutionary and functionally coherent lineages. These results illustrate that CGCD provides a high-resolution, robust, and broadly applicable framework for species delimitation. In this talk, I will introduce the principles and methodology underlying CGCD and highlight our first key results using *Acinetobacter baumannii*.

Marine cryptic diversity: insight from New Caledonian Lamellariinae (Gastropoda, Mollusca)

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Marine biodiversity on Earth remains largely a mystery: estimates span from a few hundred thousand to several million species. The adoption of molecular tools in taxonomy has revealed that a substantial, though still undefined, portion of this diversity consists of cryptic species complexes. Yet we still lack solid data on their true abundance, geographic distribution, and taxonomic spread. The marine malacofauna of New Caledonia represents one of the most extraordinary concentrations of molluscan diversity in the world, placing it among the richest regions globally. However, which percentage of this fauna is hidden by cryptic species is still to be assessed. Lamellariinae (Velutinidae), are small marine caenogastropods that possess a thin shell entirely enveloped by a large fleshy mantle. Due to lack of diagnostic shell characters and high phenotypic plasticity their diversity has long been underestimated. Thanks to several MNHN campaigns in New Caledonia and surrounding areas we assembled a genetic dataset (cox1, 16S rDNA, ITS2) of 150 lamellariine specimens, including live recorded mantle coloration patterns. Toward an integrative taxonomic approach, including radula and protoconch SEM observations, in New Caledonia and surrounding areas we found 22 species of lamellariine belonging to five genera, 16 of which known only from there so far. Seventeen of these species results also new to science and showed extensive cryptic patterns within genera, making them hardly confidently identifiable without genetic data. Our findings, both on this group and on others, confirm that cryptic species are far more frequent and widespread than previously assumed, even within regions hosting an exceptionally rich malacofauna, with remarkable implications for how biodiversity is assessed, monitored, and ultimately conserved.

The role of female reproductive fluid in intra-ejaculate sperm selection and its effects on early offspring development in zebrafish

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The female reproductive fluid (FRF) is emerging as a key mediator of cryptic female choice. This fluid, which surrounds the eggs, can recognize and differentially affect sperm performance of different males, ultimately biasing fertilization outcome. FRF may also have the potential to recognize and select among sperm within the same ejaculate, and recent evidence suggests that FRF-selected sperm have indeed less DNA damage, but consequences on embryos have never been tested. In this study, we ran a pilot experiment using zebrafish (*Danio rerio*) where we explored (i) the consequences of FRF in sperm selection for offspring fitness and (ii) the role of FRF outside sperm selection (only after fertilization). (i) In the first experiment we obtained FRF-selected or not selected (random) sperm and used those sperm to fertilize the eggs. We hence compared early fitness traits in embryo fertilized with FRF-selected *versus* non-selected sperm. We found, unexpectedly, a lower fertilization success when sperm were selected by the FRF, but the larvae sired by non-selected sperm exhibited a hyperactive locomotor activity, that is similar to that observed in other studies under stressful conditions. (ii) In the second experiment, we compared embryo fitness after being exposed or not exposed to FRF, to reveal any effect of FRF on embryonic development itself independently from sperm selection. In this case, there was no effect of FRF. Our preliminary results suggest that the sperm selection by FRF may have an impact on offspring fitness and contribute to the ongoing work to our understanding of FRF as a potential medium for sperm selection with potential evolutionary and applied implications.

An ecomorphological approach to understand parasitic adaptation in triungulin larvae of blister beetles (Coleoptera: Meloidae)

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Blister beetles (Coleoptera: Meloidae; about 130 genera and 3000 species) are remarkable not only for cantharidin production, but also for the evolution of a highly specialized first instar larva, the triungulin, present in all subfamilies except Eleticinae. Depending on the taxon, these larvae feed mainly either on provisions and immature stages of bees (Hymenoptera: Apoidea) or on grasshopper egg pods (Orthoptera: Acrididae). The food source may be reached by actively crawling over the substrate or through phoresy, primarily on bees and, only in the genus *Cyaneolytta*, on Coleoptera Carabidae. These peculiar ecological adaptations appear to have evolved multiple times within the family, and triungulin morphology is thought to vary accordingly, although this correlation relationship has never been quantitatively tested. Here, we aim to test the association between ecological strategies and larval morphology across the Meloidae family within a robust phylogenetic framework. We analyzed variation in key morphological traits (including head capsule, thoracic segments, and leg appendages) using a 2D/3D geometric morphometric approach applied to a broad taxonomic dataset composed of 130 species belonging to 45 different genera. Phylogenetic comparative methods were employed to examine shape variation in relation to ecological categories (e.g., phoretic vs. non-phoretic behaviour, host type), and to assess patterns of morphological convergence and divergence. This study sheds light on the evolution of larval morphology as an adaptation to parasitism and contributes to unveiling the mechanisms adopted by insects for the exploitation of host resources.

Limits of morphological identification in Polyplacophora: an integrative approach to species delimitation

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The long acknowledged gap between catalogued and actual marine biodiversity is recently widening, as many morphologically defined taxa are being revealed as complexes of genetically distinct cryptic lineages. In groups characterized by high phenotypic variability and limited diagnostic morphological characters, taxonomy based exclusively on morphology may substantially underestimate species diversity, even in extensively investigated regions such as the Mediterranean Sea. Mediterranean Polyplacophora represent a suitable model to evaluate the impact of integrative species delimitation approaches across an entire molluscan class with an ancient evolutionary history and a robust morphology-based taxonomy. An integrative taxonomic framework was applied to a dataset of 766 specimens assigned to 21 morphospecies of Mediterranean Polyplacophora, 212 of which were collected during the MNHN CORSICABENTHOS expeditions. Molecular validation was performed through distance-based species delimitation analyses using the ASAP algorithm applied to the *cox1* marker, and through phylogenetic reconstruction based on concatenated *cox1*+16S rDNA datasets. The integration of distance-based methods and phylogenetic evidence supported the recognition of 41 putative species, uncovering extensive cryptic diversity within Mediterranean Polyplacophora. Among the 30 species involved in 10 cryptic complexes, 20 resulted broadly sympatric with at least one other lineage of the same complex, suggesting that cryptic diversity cannot be explained solely by geographic isolation. These results revealed significant incongruences between morphology-based taxonomy and molecular evidence, suggesting that Mediterranean chiton diversity is substantially underestimated. This

study highlights the importance of integrative taxonomy for accurately assessing marine biodiversity and suggests that comparable levels of hidden diversity may remain undetected across other marine groups, including fossil Polyplacophora.

Dual Paths of Adaptation in Artificial Life: From Goal-Directed Optimisation to Circular Causation

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Directional selection tends to reduce genetic variance, potentially limiting long-term evolvability. This study adopts an alife approach, employing genetic algorithms on organisms modelled as finite-state automata evolving to anticipate environmental fluctuations. We compare two scenarios: a static fitness landscape corresponding to classical goal-directed optimisation, and a dynamic landscape that simulates niche construction, in which selection pressures are endogenously reshaped through feedback between agents and their environment. At moderate mutation rates, classical optimisation leads to entrapment in local optima and a decline in population heterogeneity, as measured by ecological metrics. Conversely, under the same conditions, the self-referential regime sustains diversity, particularly in the presence of recombination. In mixed populations, conjugation-based reproduction is favoured over cloning, although purely asexual populations more rapidly approach efficient resource-use attractors. Likewise, populations with fewer internal states converge more quickly, whereas allowing the number of states to evolve leads to higher stable values and greater behavioural diversity. In the circular causation regime, the fitness gap narrows, with decreasing maximum fitness and increasing minimum fitness, resulting in a more equitable distribution of resources, as indicated also by the Gini index. Sample-entropy further shows that dynamically reshaped environments are associated with increasing unpredictability, helping to drive the entire system towards greater structural complexity. However, after an initial phase where system development appears more predictable, the course of sample-entropy seems to depend on contingent factors. These results suggest that while stable environments favour simplification, systems that actively reshape selective pressures trigger autocatalytic dynamics promoting diversity, cooperative equilibria, and potentially open-ended evolution.

Genetic evidence for a species complex in *Callinectes sapidus* (Decapoda: Portunidae)

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Callinectes sapidus (Rathbun, 1896), commonly known as the blue crab, is an invasive alien crustacean species in the Mediterranean Sea. Native to the northwestern Atlantic, the blue crab has expanded its distribution range since the early 1900s, reaching numerous European coastal areas. Its rapid spread is attributable to its high dispersal capability, as well as to both intentional and accidental introductions via ballast water. The present study aims to investigate the evolutionary history and invasion dynamics of the blue crab across both its native and introduced ranges, by increasing the number of sequences analysed compared to previous studies. More than 800 sequences, representative of the species' whole geographic distribution, were analysed using the mitochondrial gene Cytochrome c Oxidase subunit I (COI) as molecular marker. Phylogenetic and phylogeographic analyses were performed on this dataset, integrated with species delimitation approaches to clarify the taxonomic status of the species. The results support the existence of a species complex within *C. sapidus*, consisting of two main taxonomic units: one predominantly distributed in Central and South America, and the other occurring in North America and the Mediterranean basin. Additionally, a general genetic homogeneity was observed among Mediterranean blue crab populations, consistent with a recent introduction and subsequent establishment in the region, with the exception of some Turkish populations, which showed notable genetic divergence. The identification of multiple distinct evolutionary units, and thus the presence of a species complex across the blue crab's distribution range, is particularly relevant as it may have important implications for the development of monitoring and management strategies for the species in both its introduced and native areas.

Armed to the teeth: the ontogenetic variation in diet among fossil and extant hyaenas with Total Microwear Analysis

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The family Hyaenidae is nowadays represented by four species and most of them are bone-crackers specialized in consuming meat and bones. Durophagy implies several craniomandibular features such as buccolingual wide cheek teeth and strong cranial and mandibular muscular structures. Even though these morphologies are well developed in adults, juveniles likely acquire the full extent of these morphofunctional features only after the complete weaning. Here we analyse the tooth wear on deciduous teeth to test the capability of cubs to process hard food items despite the incomplete development of the craniomandibular features related to durophagy. To do so, two methodologies for dental microwear investigation were used: the Dental Microwear Analysis and the Dental Microwear Texture Analysis. We created a dataset with hypercarnivorous species (particularly felids e.g., *Panthera leo* and *Panthera tigris*) and durophagous ones (the adult individuals of extant hyaenids e.g., *Crocuta crocuta*, *Parahyaena brunnea* and *Hyaena hyaena*) to define tooth-wear patterns associated to these ecological groups. The goal was to test possible affinities of juvenile hyaenas with one or the other group and, thus, understand possible dietary shift during the ontogeny. Following the same methodology, we included two fossil species considered as fully developed bone-crackers, i.e., *Pachycrocuta brevirostris* and *Pliocrocuta perrieri*. Our results show that the abundance of perforation marks (i.e., pits), appears to be more indicative of a durophagous diet rather than linear marks (i.e., scratches), which do not differ between felids and hyenas. Notably, two distinct behavioural patterns emerge: one involving an ontogenetic dietary shift between juveniles and adults and another showing no change. This variability occurs in both extant and fossil hyaenas and may provide insights into development and social behaviour of these taxa.

An Integrative Reference Library for East African mangrove crabs: a tool for biodiversity assessment and conservation

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Mangrove forests are highly productive intertidal ecosystems that provide critical ecosystem services. They host unique faunal communities dominated by brachyuran decapods, which act as ecosystem engineers and sustain local ecological processes. Given the low functional redundancy of mangrove-associated macroinvertebrate communities, their reliable assessment is crucial for efficient management and conservation strategies. However, this is frequently hampered by difficulties in species identification. In East Africa, complex latitudinal variations in crab community composition are further obscured by pseudo-cryptic and hybrid lineages that defy simple morphological or molecular identification. Here, an Integrative Reference Library (IRL) for East African mangrove crabs is provided, combining molecular data with morphology to ensure accurate taxonomic validation. In total, 198 specimens spanning from Somalia to South Africa, the Red Sea, and Western Indian Ocean islands were morphologically examined, and sequenced for the mitochondrial Cytochrome c Oxidase subunit 1 (COXI) gene. Barcode sequences were used to delineate Molecular Operational Taxonomic Units (MOTUs) using four single-locus species delimitation methods: the Automatic Barcode Gap Discovery (ABGD), the Assemble Species by Automatic Partitioning (ASAP), the Bayesian implementation of the Poisson Tree Processes (bPTP), and the General Mixed Yules Coalescent (GMYC). The molecular data were combined with species-level morphological diagnoses, ecological and biogeographical insights, and a field-optimized dichotomic key. By integrating DNA barcodes with morphology, 38 crab species, representing 11 families, were validated. This study establishes the first IRL for mangrove-associated brachyurans along the East African coast, provides a vital baseline to evaluate the success of local restoration programs, and enables robust environmental DNA (eDNA) monitoring tools for conservation management across the region.

Genomic Surveillance of H5N1: Evolutionary Dynamics and Zoonotic Risk

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Highly pathogenic avian influenza H5N1 has emerged as a major global concern due to its rapid spread in wild and domestic birds and its occasional transmission to mammals, including humans. Its zoonotic potential and continuous evolution make genomic surveillance essential for risk assessment. In this study, we applied comparative genomics and bioinformatic approaches to explore the genetic diversity and interspecies dynamics of H5N1, focusing on its capacity for zoonotic transmission. We analysed all available HA and NA sequences from the GISAID database, obtained from avian, non-human mammalian and human hosts across diverse geographic regions. No highly specialized or host restricted clades were identified, and the overall genetic structure remained consistent with current clade and lineage classifications. Selection analyses indicated limited selective pressure on the viral segments, although strains from high density farming environments showed a slight increase in positively selected sites, exceeding levels typically observed in wild populations. Birds, especially migratory species, were confirmed as primary hosts and reservoirs, acting as vectors between wild and domestic settings. Integrating genomic data with host ecology and epidemiological context allowed us to identify geographic areas and viral lineages with elevated zoonotic risk, highlighting recurrent evolutionary patterns linked to host switching events. This study illustrates the value of bioinformatic genetics within a One Health framework, demonstrating how large scale genomic surveillance can detect early genetic signatures of zoonotic risk and support

integrated monitoring strategies for timely prevention and response to future influenza outbreaks.

Smells like team spirit: interaural gland secretion variability in Bechstein's bats

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Chemical communication is central to animal social behaviour, particularly in nocturnal species such as bats. In Bechstein's bats (*Myotis bechsteinii*), interaural gland secretions have been proposed to mediate individual and colony recognition, yet little is known about their temporal stability and social relevance. We used gas chromatography–mass spectrometry to quantify variation in the relative abundance of compounds in interaural gland secretions across years, seasons, colonies, and individuals, and examined associations with genetic relatedness and roosting behaviour. Secretion composition varied markedly among seasons and years. Although colony-specific chemical signatures were consistently detected and individuals showed short-term repeatability, we found no evidence for stable long-term individual signatures. Chemical similarity was also unrelated to genetic relatedness. Instead, bats that roosted together more frequently exhibited more similar secretion profiles, revealing a strong association between social relationships and chemical similarity. Our results suggest that interaural gland secretions constitute a dynamic chemical signal shaped by temporal and social factors rather than a stable marker of individual identity. These findings highlight the importance of accounting for temporal variation when interpreting chemical communication and provide new insight into the role of social structure in shaping olfactory signals in bats.

Genetic load and environmental factors shape morphological asymmetry in isolated populations

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Population fragmentation often leads to loss of genetic diversity, increased rates of inbreeding, and the accumulation of genetic load. Genetic load is increasingly recognised as central to population performance. However, its phenotypic expression and interactions with environmental factors remain poorly understood. Using isolated island populations of *Podarcis* lizards as a model, we examined (i) the effects of genetic drift and inbreeding on genetic load, estimated from ddRADseq data as proxies for these genome-wide properties, and (ii) how genetic load interacts with environmental factors to shape developmental instability, measured as morphological asymmetry. High-impact variants occurred at lower frequencies than variants with likely neutral or low predicted impacts, consistent with purifying selection acting against putatively deleterious mutations. In small populations, increased genetic drift associated with long-term spatial isolation was linked to lower masked load and higher realised load within runs of homozygosity (ROH), relative to more diverse populations. Morphological asymmetry in the scaling pattern of lizards was associated with higher realised and masked load, suggesting that the accumulation of expressed or partially expressed putatively deleterious mutations may compromise developmental stability. This relationship was observed only under low environmental stress, characterised by low urban cover and high vegetation density. Additionally, asymmetry was lower in areas with higher rates of tail loss, potentially reflecting intraspecific or interspecific

interactions. Overall, our findings suggest that population history and inbreeding-environment interactions influence developmental instability in natural populations.

Hidden diversity in Andean water frogs of the *Telmatobius marmoratus* complex at the northern edge of the Altiplano

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Cryptic diversity is common in Andean amphibians and remains a major challenge for taxonomy and conservation. The genus *Telmatobius* includes several poorly resolved species complexes associated with high-elevation aquatic systems across the Andes. Among them, *Telmatobius marmoratus* and *Telmatobius jelskii* have historically shown unclear taxonomic boundaries and extensive morphological variation. Here, we used an integrative approach combining mitochondrial phylogenetics, species delimitation analyses, traditional morphology, and three-dimensional geometric morphometrics to evaluate species from the central and southern Andes of Peru. We analyzed cytochrome b (cytb) sequences from populations distributed across the Altiplano and adjacent inter-Andean basins, including topotypic material of *T. marmoratus* and related taxa. Our results reveal that both *T. marmoratus* and *T. jelskii* represent polyphyletic complexes composed of multiple independently evolving lineages. We restrict *T. marmoratus* sensu stricto to the Altiplano surrounding Lake Titicaca and adjacent regions of Bolivia and northern Chile. In addition, we propose resurrect *Telmatobius pustulosus* Cope, 1878 from synonymy and recognize it as a valid species distributed across the northern Altiplano (southern of Peru). We also identify a new cryptic lineage from the Apurímac Basin, distinguished by cranial morphology, body size, and mitochondrial divergence. Phylogenetic and morphometric evidence suggests that river basins and hydrographic isolation have played an important role in the diversification of high-Andean *Telmatobius* frogs. Our findings highlight the presence of hidden evolutionary diversity in Andean aquatic systems and emphasize the importance of integrative taxonomy for understanding

diversification processes and improving conservation strategies in highly threatened amphibian lineages.