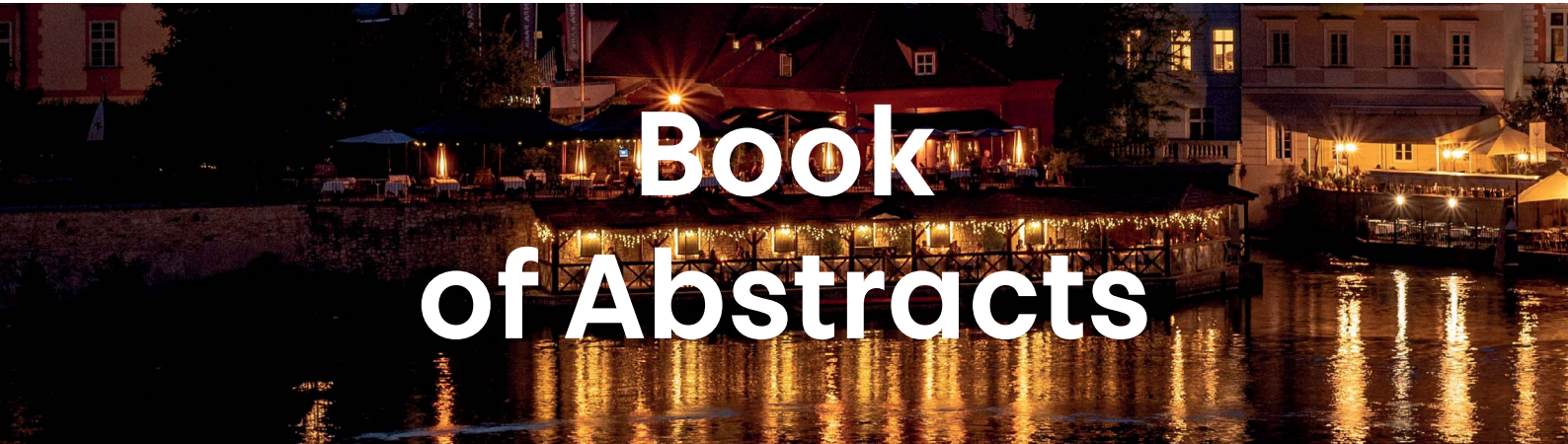




Biology of Butterflies 2023
PRAGUE, CZECH REPUBLIC
10-13 JULY 2023

The logo features a stylized butterfly with red and white striped wings, positioned above a thin orange line that represents the city skyline of Prague. The text is centered below the logo.

**Book
of Abstracts**

The background image for the bottom section shows a nighttime view of a river, likely the Vltava in Prague. The river is calm, reflecting the warm lights from the buildings and bridges along its banks. The architecture is a mix of historic and modern styles, with some buildings having balconies and outdoor seating areas. The overall atmosphere is cozy and inviting.

Biology of Butterflies 2023

Book of Abstracts

Prague, Czech Republic

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Abstracts presented at this conference have been reviewed by members of the Scientific Committee. However, the contents in the abstracts are entirely the responsibility of the author(s) concerned and do not necessarily represent the views of the organisers of the conference.

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ORAL PRESENTATIONS

Plenary talks

A global phylogeny of butterflies illuminates their evolutionary history, ancestral hosts, and biogeographic origins

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Butterflies are perhaps the most charismatic and intensely studied of all insects, serving as model and muse for dozens of seminal biodiversity studies. By leveraging methodological advances and a global network of lepidopterist colleagues, we inferred a robust molecular phylogeny of butterflies with ~2300 species from *ca.* 92% of all described butterfly genera using 391 protein-coding loci. Phylogenomic analyses produced statistically robust and congruent results that resolved previously controversial relationships. We dated the origin of butterflies to the Late Cretaceous, ~100 million years ago (Ma), and most extant family crown groups originated before the K/Pg extinction event. We aggregated larval host datasets and global distribution records from hundreds of sources and found that butterflies likely first fed on Fabaceae and originated in what is now the Americas. After most extant lineages dispersed to the Eastern Hemisphere across the Bering Land Bridge, they spread throughout the equatorial tropics in the Early Cenozoic. We examined several predictions regarding butterfly-plant coevolution and found that butterfly diversification followed radiation of their host plants, suggesting that plants were a template on which butterflies diversified. Grass-feeding skippers (Hesperiidae) and satyrs (Nymphalidae: Satyrinae) comprise nearly a quarter of all species and diversified rapidly during the Oligocene and Miocene when global grasslands expanded. This phylogenomic study provides an important scaffold for future comparative analyses of butterflies.

One wingbeat at a time: Restoring butterfly habitats and populations in the Czech Republic

Ondřej Sedláček

Ochrana fauny České republiky & Charles University, Prague, Czech Republic

Butterflies represent a well-studied group of animals in the Czech Republic, thanks to a wide entomological community comprising both professionals and amateurs. Comparing recent data on species distribution with data from the 20th Century reveals a rapid decline in many species. A number of formerly common and widespread species teeter on the edge of extinction, if not already extinct. Regional species loss and homogenization of butterfly communities are primarily caused by land-use abandonment and the intensification of agriculture and forestry. Using evidence-based case studies, this presentation summarizes conservation actions in the Czech Republic that have led to the restoration of butterfly habitats, including hotspots, as well as common landscapes and (sub)urban areas, over the past decades. These actions involve the emulation of traditional farming and forestry practices such as mosaic mowing, extensive pastures, pollarding, and coppicing, as well as the implementation of novel management techniques such as disturbance regimes utilizing fire, off-road vehicles, and motorbikes, and rewilding efforts involving the reintroduction of European bison and de-domesticated forms of cattle and horses. Additionally, several examples of butterfly reintroductions into restored habitats, which have now become suitable for the return of extirpated species, will be presented. By these steps, we aim to reverse the decline of butterfly populations and restore their habitats, one wingbeat at a time.

Extreme adaptations, phenotype, geometry, and other stories based on Lepidoptera

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Adaptation is a central concept of evolutionary biology that explains why organisms adapt to their environment under natural selection. An adaptation could be defined as a novel trait or behavior that appears in an organism and is preserved by natural selection. Limits to adaptation are a crucial aspect of evolutionary responses to any extreme environment which nowadays are influenced by anthropogenetic change. Adaptive patterns in butterflies can be both genetic and phenotypic, the latter occurring through a complex network of interactions between the genotype and the environment, and to understand these relationships, the combination of genetic, genomic, and phenotypic methodologies are completely essential which will allow us to elucidate the evolutionary origins of those adaptation in detail. This conference aims to illustrate some research made in South America where the adaptive patterns organisms are taken to extremes, a first approach in order to identify the possible mechanisms used by *Vanessa carye*, a migratory butterfly with routes from Venezuela to Tierra del Fuego, traveling almost all natural biomes with the exception of Antarctica, and some other approaches taken to the limits of altitude (5200 masl) in Andean butterflies, where the study of adaptations to extreme climates is combined with the use of methodological tools to evaluate adaptations from a morphological component using geometric morphometrics to a molecular view using population genetics.

Funding: ANID – Programa Iniciativa Científica Milenio – ICN2021_002.

The drivers of biodiversity in the Neotropics: insights from mimetic clearwing butterflies

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Interspecific interactions represent a strong driver of biodiversity structure and evolution, yet their impact at the macroecological and macroevolutionary scale is rarely evaluated. Intra-guild mutualistic interactions, where individuals from different species benefit from coexisting with each other, are particularly overlooked. Müllerian mimicry, where defended, aposematic species locally converge on a common warning signal, is a relevant system to examine the macroecological and macroevolutionary consequences of mutualistic interactions, because species that interact mutualistically have similar warning signals, thereby making mutualistic interactions relatively straightforward to characterize. The diverse Neotropical butterfly tribe Ithomiini (Nymphalidae) numerically dominates butterfly communities and is the largest clade of mimetic butterflies. One intriguing feature of Ithomiini, also known as clearwing butterflies, is that most species have partially transparent wings, which raises the question of the drivers of the evolution of such a trait in the context of aposematism and mimicry. Here I present the results of over 15 years of collaborative research on Ithomiini, which highlight that 1) mutualistic Müllerian mimicry is a strong driver of community structure, 2) mimicry has driven an evolutionary association between warning colour pattern and multiple facets of the ecological niche of species, both at the local and global scales, which 3) may have fueled the diversification of Ithomiini. I also present recent results on the evolution of transparent wing patterns in the context of aposematism and mimicry, which show that transparency is involved in both crypsis and mimicry, and that clearwing ithomiine species likely make the best of both worlds.

Systematics and biogeography of Lepidoptera: pattern and process

KEYNOTE TALK

Bridging macro- and microevolutionary studies in Neotropical butterflies

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Research programs unifying evolutionary and ecological studies in butterflies are urgently needed to understand the processes generating species diversity patterns. We focus on one of the largest radiations of neotropical butterflies, the Skipper subfamily Eudaminae (Hesperiidae). We use high-throughput DNA sequencing and ecological experiments in the lab and in the field to shed light on how multiple strategies to survive predators explain the extant Eudaminae distribution and diversity patterns. First, we provide evidence that the large-scale phenotypic convergences in wing shape (hindwing tails) along the phylogeny of Eudamini might be explained by selective pressure exerted by avian predators. Such morphological cues might advertise prey unprofitability, for example, unpalatability or escape ability. Second, upon investigating further in a rainforest locality in Peru, we found evidence that indeed a combination of evasiveness and unpalatability might explain their strong unprofitability to local insectivorous birds, providing an explanation for the high phenotypic evolution and species diversification rates along its phylogeny. Third, by integrating phylogenetic and population coalescence processes, we revealed species diversification patterns, including lineages that have radiated rapidly and recently, and others that have gradually accumulated over millions of years. Further, intrinsic barriers to gene flow seem to be strong in this group given that closely related, syntopic *Spicauda* species in localities in Peru and Panama have remained strongly differentiated. Altogether, we are starting to reveal how extant neotropical butterfly species, genetic, and functional (unprofitability signaling) diversity have been shaped by evolutionary and ecological mechanisms.

Where? When? How? Patterns and processes on the biogeographic history of Acraeini butterflies

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Historical biogeographic studies on pantropical disjunctions of several butterflies groups have been explained mainly by the direct long-distance transoceanic dispersion (LDD), with subsequent speciation. That is the explanation suggested for the nymphalid butterflies of the tribe Acraeini (Heliconiinae), found throughout the tropics, with higher species diversity in the Afrotropical region. Recent data on this group at different taxonomic levels revealed that the Acraeini lineage diversified in the end of the Eocene at 37.4 Ma. Three possible LDDs took place, one from Ethiopian to Australian region (about 16 Mya), another on the ancestor of *A. camaena* that went back to the Ethiopian region, and a third one from Ethiopian to the Andean region during the Oligocene, an event that gave origin to the Neotropical lineage. In the Neotropics the group reached the Atlantic Forest during the mid-Miocene (crown age of *Actinote*: 10 mya), and at least two very recent dispersion events in Quaternary ice ages (2.5 to 0.01 Mya) occurred within the genus *Actinote* from the Atlantic Forest to the Amazon basin, throughout connections by gallery forests in the dry diagonal area between these biomes. Several different processes of diversification took place in the Atlantic Forest, including montane speciation, and phylogeographic structure recovered with genomic data of montane species confirms the role of disjunct distribution on different mountain ranges in the population genomic structure in Acraeini butterflies of the Montane Atlantic Forest, associated to the São Paulo subtropical gap as a phylogeographic break.

Diversification and biogeography of pantropical grass yellow butterflies (Pieridae: Coliadinae)

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Grass yellow butterflies are a group of pantropical pierid genera (*Abaeis*, *Eurema*, *Leucidia*, *Pyrisitia*, *Teriocolias*) distributed throughout the Old and New World tropics. Little is known about its diversification pattern, including the biogeographic mechanisms explaining their global distribution. We present the first densely sampled, time-calibrated phylogeny and biogeographic reconstruction to confirm the monophyly of the genera and widespread species. Additionally, we inferred the biogeographic events attributing to their current, cosmopolitan distribution. We analyzed a combination of published butterfly sequences and newly generated data via anchored hybrid enrichment to infer Bayesian and maximum likelihood phylogenies with divergence dates. We fitted alternative biogeographical models and used the best fitting model to estimate relative dispersal events through time using biogeographical stochastic mapping. Our phylogenies recovered similar topologies with moderately good support for Old and New World *Eurema*, except for one clade containing Cuban endemic grass yellows. Several widespread species were not monophyletic. *Eurema* emerged ~26 Mya, dispersed to the Old World ~23 Mya, and subsequently diversified there ~20 Mya. We recovered paraphyletic relationships for New World *Eurema* in all trees, especially to *Pyrisitia*, *Abaeis*, *Leucidia*, and *Teriocolias*. Moreover, our biogeographic analysis suggests that South America and Sundaland are sources of dispersal within the Old and New World, respectively. Overall, our results confirm that New World *Eurema* is polyphyletic whereas Old World *Eurema* are monophyletic. Their global distribution may be best explained by a single dispersal event across Beringia.

Phylogeny of the neotropical subtribe Pronophilina (Nymphalidae, Satyrinae) based on target enrichment and adult morphology

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For the first time a comprehensive phylogenetic hypothesis of the subtribe Pronophilina, the most diverse group of Neotropical montane butterflies with least 650 species, is presented based on target enrichment data of all recognized genera (except monobasic *Paramo*) and 80% of species, supported by comprehensive morphological data, with an emphasis on genital structures. It is confirmed that the group is monophyletic, fully supported, and sister to the subtribe Euptychiina. Seven major clades are recognized: *Pronophila*, *Pedaliodes*, *Lymanopoda*, *Manerebia*, *Eretris*, *Steroma*, and the infratribe Neosatyriti. Previous hypothesis suggesting closer relationships of the Neosatyriti with the Australian Hypocystina, and the position of *Manerebia* within the Holarctic Erebiina are rejected. Our study confirms the validity of most of the genera described so far, except for some in the *Pedaliodes* clade, i.e. *Altopedaliodes*, *Neopedaliodes*, *Dangond* and *Pherepedaliodes*. Within the *Pronophila* clade, two new genera are described, *Boyeriana* and *Fahraeusia*, whereas the genus *Arhuaco* is invalidated. The subtribe evolved simultaneously with the uplift of the Andes, with the earliest divergence phenomena taking place in the Oligocene, some 26 mya. Most extant genera arose in the Pliocene. During the Pleistocenic glacial epochs an intense radiation took place especially within the *Pedaliodes* clade, with both allopatric and parapatric speciation along altitudinal gradients. The centre of diversity of the tribe corresponds with the eastern slopes of the Andes in Central Peru. The subtribe first colonized Mesoamerica with the formation of the Isthmus of Panama some 3 mya, approximately simultaneous with the first colonization of the Guyana Shield.

Wing pattern, host plant use and genome skimming data suggest speciation in the Caribbean Metalmark, *Dianesia carteri* (Lepidoptera, Riodinidae)

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The family Riodinidae is mainly distributed in the world tropics but is highly diverse only in the Neotropics. However, only one endemic monotypic genus, *Dianesia*, represents the family in the Caribbean Islands. *Dianesia* is restricted to the Bahamian Islands and Cuba where the nominal subspecies and the subspecies *ramsdeni*, respectively, are traditionally recognized. We assess the status of these taxa by analyzing specimens from populations scattered across both archipelagos. We compare the wing pattern of 70 available specimens, using the R packages *patternize* and *recolorize*, and apply a genome skimming protocol to 9–29 year old samples from several populations. For the majority of the samples we obtained most of the mitochondrial genome and 1066 orthologous nuclear loci. We performed population analyses in the *Structure* software, and inferred a maximum likelihood phylogeny in *IQTREE2* to have an insight into the possible diversification within *Dianesia*. We interpret the results together with recent host plant information indicating that some *Dianesia* populations use heavy metal hyperaccumulator host plants while others do not. The available information suggests that *Dianesia* instead of being monotypic comprises at least five species.

Habitat opening fostered diversity at both continental and local scales: impact of dispersal and habitat-shifts in the diversification of a speciose Afrotropical insect group

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The opening of habitats during the Neogene was a significant event that impacted the evolution of biodiversity, particularly in Africa where the largest surface of grasslands and savannas is located. Its influence on species diversification may be investigated at large scales by examining extrinsic factors (Court Jester model; CJ) and at small scales through the consideration of more intrinsic biotic interactions (Red Queen model; RQ). Here, we investigated the evolution of noctuid stemborers (Lepidoptera: Noctuidae: Sesamiina), a group of moths mostly associated with open habitats, and which are most diverse in the Afrotropics. We generated a dated molecular phylogeny for 229 stemborer species and further assess the role of habitat opening on the evolutionary trajectory of the group through parametric historical biogeography, ancestral character state estimation, life history traits and habitat dependent diversification analyses. Our results support an origin of stemborers in Southern and East Africa *ca.* 20 million years ago, with range expansions linked to the increased availability of open habitats that acted as dispersal corridors. At a local scale, the initial specialization on open habitats was maintained over time, with shifts towards closed habitats rare and unidirectional. Analyses of life history traits show that habitat changes led to or at least preceded specific adaptations, such as changes in larval behavior and color. By comparing these findings to those previously inferred for *Bicyclus* butterflies (Lepidoptera: Nymphalidae: Satyrinae) which are mainly distributed in closed habitats, we have recovered almost opposite patterns of diversification, both on a continental and local scale.

Neotropical montane butterflies: combining phylogeny, ecology and biogeography to understand patterns and processes

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The genus *Actinote* (Nymphalidae: Heliconiinae: Acraeini) includes 38 species of Neotropical butterfly most associated with montane forests. In recent years, efforts were made to compile all available data in order to understand the patterns of biodiversity and biogeography in this group. A recent dataset of more than 3000 geographical records and a recent phylogenetic hypothesis to 30 species served as basis to solve several of the above questions. As previously mentioned, the genus is mostly associated with medium to high altitudes, with very few species present in the lowlands (such as the Amazon basin). Based on the biogeographic history of the group, the genus *Actinote* originated and diversified in the Atlantic Forest, where the maximum species richness (up to 18 sympatric species) and phylogenetic diversity are found. The Andean region was colonized several times, but local diversification was low, resulting in a lower species richness, but high phylogenetic diversity. Based on paleoclimate modelling, a south corridor during periods of low temperatures (associated with Pleistocene glaciations) was suggested as the more plausible route from the Atlantic Forest to the Andes; however, no colonization in the reverse direction has been inferred. The present results show the importance of combining different sources of evidence to reveal patterns and processes in the evolution of butterfly groups, adding important information to understand the genesis of the high Neotropical biodiversity.

KEYNOTE TALK

Insights from large-scale butterfly genomics

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The technological revolution enables scientific advances. We capitalize on the recent breakthroughs in sequencing methods to obtain whole genome datasets for many thousands of specimens, from recently collected to two centuries old. Using butterflies as model organisms, we address general problems in biology, from learning about genotypic determinants of phenotypic traits and associating proteins with morphology to revealing principles of evolution in animals and the significance of gene exchange between different species through hybridization. We use genomics to solve long-standing problems in nomenclature, such as associating century-old and frequently damaged primary type specimens with present-day populations and deducing type localities. Genomic datasets yield well-resolved phylogenies and improve the taxonomic classification. Genome-scale trees reveal levels of diversifications, i.e., periods of bursts followed by extinctions, suggesting that a limited number of taxonomic ranks, such as tribes and genera, can be applied to these levels defined by longer tree branches. This apparent discreteness resulting from continuous processes is observed at all levels, including species. We use genomic analysis to learn about species boundaries and assist in species delimitation. Examples from our most recent work to illustrate these general principles will be shown.

So familiar, yet so unknown: Taxonomy and DNA barcoding of the Palearctic *Papilio machaon* species-group

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Swallowtail butterflies of the *Papilio machaon* species group provide a diverse and ideal model system to study hybridization. Species limits and systematic relationships have been notoriously difficult to resolve in this group in part due to a plethora of ecological races, color morphs, and incomplete reproductive barriers, as well as abundant natural hybridization. Although the evolution of the North American species has been studied extensively, studies on the Old World species are lacking. In the Palearctic region, four species are currently recognized within the *P. machaon* species complex: *P. machaon*, *P. saharae*, *P. hospiton* and *P. xuthus*. Their distributions vary, some are widespread while others are more localized. Some of the species hybridize while others do not. Current environmental changes are modifying species distributions and consequently their biotic interactions. Studies have shown that, under future climate scenarios, species distributions will shift and shrink considerably. This can alter the gene flow between species and even cause extinctions. An adequate understanding of the hybridization processes is of utmost importance in predicting the effects of rapid environmental changes to species survival. In order to shed light on the taxonomy and evolution within the *P. machaon* species group, the mitochondrial cytochrome c oxidase subunit I (COI) gene was sequenced for 264 *P. machaon*, 5 *P. saharae* and 20 *P. hospiton*. Initial results reveal a complex genetic pattern consistent with the response of the species-group to the last glacial periods. A taxonomic revision of the species-group is proposed.

High altitude isolation and speciation of butterflies in the Irano-Turanian mountain plateaus

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Even though the high plateaus of Qinghai-Tibet and Iran share many faunal elements, the historical biogeography of the species present in this area are not very well understood. Our investigations into the radiation of various butterfly groups present in this region unveil presence of distinct mitochondrial lineages that do not always correspond with morphological differentiation between populations. I will present several case studies that all show common patterns of haplotype diversity, shedding light on poorly understood climatic events in recent geological history that shaped the current butterfly fauna in this region.

The genetic legacy of the Quaternary ice ages for West Palearctic butterflies

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It is widely accepted that the interplay between climatic oscillations over the last 2.6 million years and geographic barriers had a central role in shaping genetic variation of the European biota. Two landmark papers proposed a generalisation of this phenomenon —the Taberlet-Hewitt hypothesis— but a comprehensive data-driven assessment has never been attempted. Using West Palearctic butterflies as a model, we applied novel comparative landscape genetic methods to a massive dataset comprising ~30,000 COI sequences derived from 505 species. This allowed us to build an objective model of the genetic legacy of the Quaternary ice ages, covering Europe, North Africa and the Middle East. Our analysis retrieves eight bioregions generalising the evidence for glacial refugia in the Maghreb, Iberia, Italian Peninsula and Balkans and also providing evidence for extra-Mediterranean refugia around the Alps and Carpathians. We also document latitudinal gradients in genetic variation and haplotype endemism, confirming the 'southern richness, northern purity' hypothesis. These latitudinal trends were mostly caused by higher fragmentation among bioregions in the south, confirming allopatric divergence as a main determinant of genetic differentiation. By moving from case studies to the statistical analysis of an entire biotic assemblage, we also identified main postglacial dispersal routes and contact zones demonstrating the direct effect of Quaternary ice ages on intraspecific genetic variation and shedding light on the historical dynamics of genetic diversity. This approach can be used to expand knowledge of other taxonomic groups and is scalable to eventually encompass life on Earth.

Genetic structure, phenotype and evolutionary history of butterflies in the European *Polyommatus eros* species group

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The *Polyommatus eros* species group is one of the little studied and taxonomically debated Eurasian butterflies. In Europe, two or three species (*Polyommatus eros*, *P. eroides* and *P. menelaos*) were traditionally recognized by most authors. More recent works and European checklists list only *P. eros*, probably based on relatively recent molecular data showing no or little divergence among taxa within this species group. However, such published molecular data mainly consist of DNA barcodes and, for a few specimens, one nuclear marker (ITS2).

We used ddRAD sequencing coupled with analyses of spectral properties of male wings to study the genetic structure, phenotype and evolutionary history of European populations belonging to this species group.

Our results support the close relationship of taxa within this species group and revealed a genetic structure that generally matches the reported distribution of taxa *P. eros*, *P. eroides* and *P. menelaos*. Interestingly, *P. menelaos*, endemic to the Peloponnese and less often regarded as a species, was recovered as the most genetically diverged taxon, sister to all the rest in the group, and displayed almost no admixture. Analyses of wing spectral properties confirmed that *P. menelaos* is the most differentiated taxon of the group and can be distinguished from *P. eroides* based on the spectral position of the reflectance maxima; while statistically significant differences were found between all the taxa examined.

This study showed that *P. eros* and *P. eroides* are closely related, but highlights the uniqueness of *P. menelaos*, which may be considered as a species.

A new *Melitaea* butterfly endemic to the Balkans revealed by genomics

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The species pair *Melitaea athalia* and *M. celadussa* represents one of Europe's best known examples of parapatric taxa showing intermediate male genitalia characters across a relatively narrow contact zone ranging from the Pyrenees to the western and central Alps.

However, recent DNA barcode data revealed the presence of a well-differentiated and nearly sympatric clade within *M. athalia*, apparently restricted to the Balkans. Further genomic studies focusing on the contact zone between *M. athalia* and *M. celadussa* also highlighted the differentiation of the Balkan lineage corresponding to DNA barcode data, suggesting potential cryptic diversity within *M. athalia*.

Here, we used double digest restriction site-associated DNA sequencing (ddRADseq) to further investigate evolutionary relationships and species boundaries within *M. athalia*. To provide better context, we also included most of the European taxa belonging to the *athalia* group.

All analyses performed (phylogenetic trees, species trees, STRUCTURE, PCA) recovered the Balkan clade of *M. athalia* as clearly distinct and sister to *athalia* and *celadussa*. Notably, sympatry between *M. athalia* and its Balkan lineage has been detected at two Albanian sites based on two pairs of specimens. Both cases showed virtually no admixture. No mito-nuclear discordance was detected in specimens of the Balkan clade, suggesting that DNA barcodes may represent an effective way of documenting the distribution of this lineage.

These results indicate that the Balkan lineage of *M. athalia* likely represents a cryptic species apparently restricted to the western Balkans, which we name *M. balcanica* sp. nov.

Butterflies as ghosts of the mammoth steppe

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The Pleistocene cold mammoth steppe, roamed and sustained by large herbivores, was the most extensive biome on Earth reaching from Spain to Alaska. It represented a connection of tundra and steppe mammalian assemblages and consisted of a fine mosaic of various habitats. Based on molecular markers and species distribution models, we studied the biogeographic history of 30 butterfly species differing in habitat specialization through their whole distribution range (Palearctic or Holarctic). We show that in large parts of the range (Eastern Europe and Asia), most species linked to open habitats lived in vast areas during both glacials and interglacials. Cold-adapted and continental grassland butterflies formed joint communities similar to the Pleistocene mammals. Many open habitat butterflies are probably primary glacial specialists, but could have survived the short interglacials thanks to the habitat management of megafauna. In separate parts of their range, species reacted differently to Quaternary climate changes, with retreats from glaciated parts in Europe and North America. In Eastern Europe and Asia, species tend to form wide longitudinal lineages, which recolonized the deglaciated areas. Our results indicate that the open habitat butterflies prefer heterogeneous landscapes created by large herbivores. Recent homogenization of landscapes might result in poorer ability of butterflies to react to climate change by tracking their preferred habitats.

Intra-specific transatlantic dispersal and species displacement explain Holarctic disjunct distributions in *Vanessa* butterflies

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Biogeographic disjunctions have long puzzled evolutionary biologists and biogeographers. Long-distance dispersal may shape the onset of intra-specific disjunct distributions and thus the early stages of allopatric speciation, ultimately leading to inter-specific disjunctions. *Vanessa* butterflies display a wide array of migratory behaviors, from low dispersive species with restricted distributions to widespread species carrying annual migratory cycles. Hence, the biogeographic history of this genus is intriguing, with cases of both intra- and inter-specific disjunctions. *Vanessa atalanta* is present in the Nearctic and Western Palearctic, but absent in Asia, and its sister species *V. tameamea* is endemic from Hawaii. *Vanessa indica* is present only in Asia and its sister species, *V. vulcania*, is endemic from the Canary Islands. Here, we investigate this conundrum through population genomics and demographic analyses of *Vanessa* species distributed in the Holarctic. Using ddRAD data from samples across the entire distributional range of *V. atalanta*, we identify two structured populations at the continental scale, isolated by the Atlantic Ocean. Demographic simulations suggest a long-distance dispersal event from the Nearctic to Europe around the LGM. When looking at potential hybridization between Holarctic species, we found evidence of introgression between the Palearctic *V. atalanta* population and *V. indica* + *V. vulcania*. This is a strong indication that their distributions overlapped when *V. atalanta* colonized Europe. We hypothesize that species displacement of *V. indica* from Europe to Asia would explain the modern allopatric distribution. These results show the importance of long-distance dispersal for speciation and for shaping biogeographic patterns from species interactions.

Species boundaries of *Phyciodes* butterflies (Lepidoptera: Nymphalidae) based on genomic integrity in a region of contact

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Crescent butterflies of the *Phyciodes tharos* group have a long-standing reputation for taxonomic difficulty. We assessed species boundaries in the *P. tharos* group using genome-wide SNP data, focussing on sampling in western Canada where four species (*P. tharos*, *P. cocyta*, *P. pulchella* and *P. batesii*) have classically been recognized. Phylogenetic and cluster-based analyses confirm that there are indeed four species based on the maintenance of genomic integrity in the presence of occasional hybridization and low levels of gene flow. Mitochondrial COI does not distinguish species, with haplotypes extensively shared between species. Limited sampling of *P. batesii* indicates complex boundaries and interactions with other *Phyciodes*, especially with *P. pulchella* and *P. cocyta*. This study demonstrates the power of a genome-wide perspective to elucidate the integrity of boundaries between recently diverged species that remain capable of occasional hybridization.

The evolutionary history of Lepidoptera: where are we?

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Lepidoptera are one of the big 4 orders of insects, with some 160,000 described species, and probably a similar number awaiting discovery. Yet, compared to the other diverse orders of insects, it appears to be relatively young, with a fossil record starting some 200 million years ago. In my presentation, I will be reviewing our knowledge of the evolutionary history of Lepidoptera, and highlighting the conflicts in information from molecular data compared to the fossil record. Because we are still unsure about the timing of diversification of Lepidoptera in general, many conclusions that are found in the literature regarding the evolutionary history of Lepidoptera can be questioned.

Inferring species-level phylogenies with whole-genome data: a new step in deciphering the taxonomy and history of the recalcitrant tribe Leptocircini (Papilionidae)

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Leptocircini is a dazzling tribe of Papilionidae, including dragontails, kite swallowtails, and swordtails. This tribe enjoys a wide distribution notably throughout the tropics of Africa, Southeast Asia, and the Neotropics, making it a fascinating subject in evolutionary biology. Yet, despite representing a quarter of the global swallowtail diversity, Leptocircini have been neglected in recent global phylogenetic analyses, leaving unanswered questions about its taxonomy (number and validity of species) and systematics (number of subgenera, genera, and the relationships between them). Here, we present a new taxonomic working list for Leptocircini, featuring 156 valid species. Using long and short reads whole-genome data, we produced new reference genomes, and generated 149 highly covered and scaffolded whole genomes to infer densely sampled phylogenetic hypotheses. Based on mitochondrial or thousands of nuclear genes and multiple phylogenetic approaches, a robust phylogenomic tree representing >93% of Leptocircini is recovered. We confirmed or explored some of the key phylogenetic hypotheses, such as the placement of the monotypic genus *Protographium* as sister of all *Graphium*. Additionally, we found that subgenera *Paranticopsis* and *Pathysa* were paraphyletic and that the most recently proposed structure of *Eurytides sensu lato* (*Neographium*, *Mimoides*, *Eurytides Protesilaus*) needs revision, as *Neographium* is paraphyletic with *Mimoides*. Our analyses also raise concerns about potential taxonomic inflation in some species groups, such as the *adamastor*, *sarpedon* and *Protesilaus* species groups. Overall, our study sheds light on the clade's macro-evolutionary history and paves the way for further research on this diverse and charismatic tribe of butterflies.

Genomics reveals the genetic structure and cryptic diversity in *Pyrgus armoricanus*

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The widespread species *Pyrgus armoricanus* has been traditionally regarded as comprising two main subspecies (*P. a. armoricanus* and *P. a. persicus*), a division mostly based on differences in male genitalia. The former taxon is found in Europe west of the Balkans, while *P. a. persicus* is known from south-eastern Europe and Asia Minor to Iran. Recent research on mtDNA variation detected intraspecific divergence over 2% in sympatry in areas where specimens putatively belonging to the two taxa meet (Balkans and eastern Europe). This level of differentiation, coupled with reported differences in male genitalia, raises the question whether the two taxa represent different species.

In this study we analyzed genomic data of *P. armoricanus* obtained with double digest restriction-site associated DNA sequencing (ddRADseq). Specimens were sampled throughout the southern and eastern European range to Turkey and Armenia. The genetic analyses suggested the presence of a “western” and “eastern” division within *P. armoricanus* with admixed specimens at the contact zones (Romania and Ukraine). The specimens with over 30% of “eastern” ancestry had “eastern” mtDNA haplotypes, while the rest had “western” haplotypes. At the chromosome level, the highest differentiation between the two groups was observed on the Z-chromosome. Based on ddRADseq reads, *Wolbachia* infection appeared to be almost exclusively restricted to the “eastern” clade, which seems to be heavily infected.

The results, including the presence of a narrow and asymmetric area of admixture, suggest the presence of isolation mechanisms that match the pattern of other sister cryptic species across Europe.

Opening up a treasure trove of color patterns and life histories: Phylogenetic relationships within the superfamily Zygaenoidea based on museomics

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The Zygaenoidea contains over 2600 described species currently in 13–15 mainly smaller families with a worldwide distribution. The variation in morphology and life history is immense. Many are day-flying, very colorful, often with aposematic patterns and/or involvement in mimicry rings, and would be highly interesting for studies on color pattern and life history evolution, and the genes behind these. Such studies are hampered by lack of knowledge. We know very little about the phylogenetic relationships of these families. There has been great fluctuation in the systematics, and taxa have been moved around between families within the suborder, but also out of the suborder all-together, and others have been moved in, often from very distantly related clades. There has been some Sanger-based molecular work as part of larger Lepidoptera studies, and some work on the Zygaenidae and Limacodidae, but only very few zygaenoid taxa have been included in any larger phylogenomic analyses due to lack of fresh material. Here we present the first preliminary higher-level phylogeny of the superfamily Zygaenoidea using a combination of target enrichment and low-coverage whole genome sequencing data based on museum specimens. We show that several families are para- or polyphyletic, and at least one new family needs to be erected. Additional work on the phylogenetic relationships in the Zygaenidae and Limacodidae will be presented, leading the way to further studies on color pattern and life history evolution.

Open topic: the diverse traits and processes in Lepidoptera biology

KEYNOTE TALK

Shifting balances in the weighting of sensory modalities are predicted by divergence in brain morphology in incipient species of *Heliconius* butterflies

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Integrating and weighting sensory perception across modalities is crucial to how animals adapt to their environment. Divergence in brain structure is often in sensory processing regions, suggesting that investment reflects ecological needs. Here, we use two parapatric closely related species, *Heliconius erato cyrbia* and *Heliconius himera*, to test the hypothesis that divergence in sensory brain regions affects foraging decisions. These butterflies are isolated across an ecological gradient, which is linked to differences in brain morphology, with *H. e. cyrbia* investing more in visual centres and *H. himera* investing in olfactory centres. Here, we demonstrate that these two species vary in how they associate visual and olfactory cues with positive food rewards. We found that when individuals were trained on paired olfactory and visual stimuli, then presented with these stimuli in conflict, they showed distinct behavioural responses. *Heliconius himera* was more likely to favour positive olfactory cues than *H. e. cyrbia*, which favoured visual cues regardless of the paired stimulus. This suggests that these species have diverged in the emphasis placed on these different sensory domains during foraging, consistent with observed differences in brain morphology. This result strengthens evidence that speciation initiated by local adaptation is partly facilitated by changes in the neural basis of key behavioural functions.

Gliding in the Amazonian canopy: adaptive evolution of flight in *Morpho* butterflies

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Butterflies display extreme variation in wing shape, colour and flight behaviour associated with a tremendous ecological diversity. Despite the central role that flight likely played in their diversification, the selective pressures shaping the diversity of flight behaviours and morphologies of butterflies remains largely unknown. We studied the adaptive codivergence in wing shape, flight behavior, and aerodynamic efficiency among *Morpho* butterflies living in different forest strata by combining highspeed videography in the field with aerodynamic simulations. We showed that microhabitat specialization has resulted in the adaptive divergence of flight behaviour and wing shape, opposing efficient gliding flight in species living in the canopy to powerful flapping flight in species living in the understory. We further investigated the influence of microhabitat on the evolution of performance during evasive climbing flight, revealing that canopy species also achieve higher climbing efficiency through changes in wingbeat kinematics and morphology. By combining biological and physical approaches that bring together naturalistic observations, analyses of flight behavior and aerodynamic modelling, our study demonstrate the role of morphology in the adaption to a novel habitat — the canopy — whereas such a role in most often only postulated.

Lepidopteran prolegs are not leg homologs

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Lepidopteran larvae have both thoracic legs and abdominal prolegs, yet it is unclear whether these are serial homologs. A RNA-seq analysis with various appendages indicated that the proleg transcriptome mostly resembles the head-horn transcriptome, a novel trait in the lepidoptera, but not a thoracic leg. Under a partial segment *abdominal-A* knockout, both thoracic leg homologs (pleuropodia) and prolegs can develop in the same segment, arguing that both traits are not serial homologs. Further, a coxal marker gene, *homothorax*, was absent from prolegs but an endite marker gene, *gooseberry*, was expressed in prolegs, suggesting that prolegs may be using a modular endite GRN for their development. We propose that larval prolegs are atavistic traits derived from the re-activation of the endite GRN of primitive abdominal limbs.

Testing the role of flight in the evolution of butterfly wing tails: a combined macro-evolutionary and experimental analysis

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The evolutionary drivers of the stunning diversity of butterfly wing size and shape are poorly understood. While flight is central to many behaviours of critical importance for fitness, like courtship, migration or predator avoidance, the precise link between wing shape and flight performance has been scarcely studied. Butterfly wing tails in particular, are a striking morphological structure whose evolutionary drivers have received little attention. Their repeated independent evolution in a wide diversity of groups points at an effect of selection, but the nature of this selection is unclear. What is the importance of wing tails for flight? It was suggested that tails may deflect predators' attacks, but what happens to the butterfly when the tails are lost? Is there any aerodynamic cost to attack deflection? To investigate these questions, we combined a large scale analysis of wing shape evolution across the Papilionidae family to an experimental analysis of the flight of *Iphiclides podalirius*. Our experimental results suggest that tails affect flight, by increasing acceleration and flight stability. At the macroevolutionary scale, the presence of wing tails affects the evolution of the forewing shape, also consistent with an impact on flight. Butterfly wing tails evolution might thus result from a trade-off between predation deflection and flight performance.

Ejaculates in Lepidoptera: a hidden diversity

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The ejaculates represent a key aspect of the animal reproductive strategy. It is widely accepted that in butterflies and moths, during copulation, males transfer nutrient-rich spermatophores that are digested by the females in the bursa copulatrix. However, the actual content of lepidopteran ejaculates, as well as their adaptive value, are far from being known in detail. We here discuss three cases in which the “nutritious spermatophore” notion is incomplete or wrong. First, we show that in the butterfly *Leptophobia aripa* (Pieridae): (a) males transfer spermatophores that are not digested by females; (b) the females lack the necessary apparatus for the mechanical digestion of the spermatophores, and (c) the spermatophores are poor in nutrients, suggesting sexual coadaptation. Second, we also show that in several prominent moths (Noctuidae) males transfer, together with the spermatophore, complex deciduous cornuti. We present data on its fine structure, shedding mechanism and inter- and intra-individual variation in numbers in one species (*Peridea anceps*). Finally, in several skipper butterflies (Hesperiidae) males transfer, besides the spermatophore, numerous relatively large granules. We present data on interspecific variation in the size and numbers of these granules and show that their composition is mostly inorganic. These lines of research suggest that the ejaculates of lepidopterans have been oversimplified and should be studied in more detail even in well-studied groups.

Infection with a male-killing *Spiroplasma bacterium* drives morphological changes in the African monarch butterfly

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Sexual selection and conflicts within and between sexes promote morphological diversity of reproductive traits within species. Variation in the morphology of diagnostic reproductive characters within species offer an excellent opportunity to study these evolutionary processes as drivers of species diversification. The African monarch, *Danaus chrysippus* (Linnaeus, 1758), is widespread across Africa. The species is polytypic, with the respective geographical ranges of the four colour morphs only overlapping in East Africa. Furthermore, some of the populations host an endosymbiotic bacterium, *Spiroplasma*, which induces son-killing and distorts the local host population sex-ratio, creating sexual conflicts. We dissected females from Canary Islands, Kenya, Rwanda and South Africa, and conducted microscopy imaging of their reproductive organs. We then characterized the effect of population, female body size, and female mating status, on the size and shape of different genitalia characters of the *D. chrysippus* female butterflies. We showed that although the general morphology of the organs is conserved, female genitalia vary in size and shape between and within populations. The small virgin females have the smallest organs, while the same organs were expanded in mated females. Females from highly female-biased populations, where the male-killing *Spiroplasma* is prevalent, also have a larger area of their corpus bursae covered with signa structures. These results suggest that male depletion due to the symbiont, might result in smaller spermatophores, and select for female genitalia features that optimize the digestion of small nutritious spermatophores

The contributions of plant chemicals and monarch genetics to resistance against a virulent protozoan parasite

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Tolerance and sequestration of toxic plant chemicals from larval host plants are common evolutionary strategies within Lepidopterans for diversifying diet and fighting predators and parasites. Monarch butterflies (*Danaus plexippus*) consume toxic chemicals, called cardenolides, from milkweed host plants (genus *Asclepias*), which provides resistance against their virulent protozoan parasite, *Ophryocystis elektroscirha*. However, parasite resistance varies between monarch populations, and it is unclear how milkweed chemistry and monarch genetics may be interacting to shape these differences. We experimentally tested the roles of cardenolides and monarch genetics in interactions between monarchs, milkweeds, and parasites. When manipulating concentration and composition of cardenolides fed to infected monarchs, we found both factors to be important in providing measurable parasite resistance, suggesting potential synergistic interactions between milkweed chemicals. Because monarch populations across the globe consume different milkweed species that vary chemically, we also tested parasite resistance in monarchs from two populations, Eastern North America and Puerto Rico, reared on native and non-native milkweeds. We found greater parasite resistance in Puerto Rican monarchs compared to those from Eastern North America and no effect of host plant in Puerto Rican monarchs, suggesting that this population has greater genetic resistance. This could be because parasites of Puerto Rican monarchs have adapted tolerance to cardenolides in milkweeds native to Puerto Rico. Alternatively, the loss of migratory behavior in Puerto Rican monarchs, resulting in the loss of migratory escape (a major life history strategy that reduces parasite pressure), could have increased parasite pressure, thus selecting for genetic resistance within Puerto Rican monarchs.

Colorful genetics of life history traits in Lepidoptera

KEYNOTE TALK

On the costs and benefits of pteridine co-option into Pieridae wings

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A simple explanation for the correlation between variation in color and life history traits is that colors differ in their intrinsic costs. Here I focus upon this issue in the Pieridae, bringing together historical literature and recent findings, with a focus upon the pteridine pigments characteristic of this family. Regarding historical hypotheses about the costs and benefits of pteridine formation in wings, we reinterpret our functional genomic insights into the suppression of pigment granule formation and associated fitness benefits. We also present new CRISPR results and a comparative analysis of scale structures in Pieridae, discussing their implications for pteridine co-option into their wing scales. Beyond highlighting ways that my group will be pursuing these issues, my goal is to stimulate discussion with similarly curious minds about how to probe relationship between color and life history traits for possible future collaborative efforts.

Yellow regulates courtship plasticity in butterflies

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Seasonal plasticity in male courtship in *Bicyclus anynana* butterflies is due to variation in levels of the steroid hormone 20E (20-hydroxyecdysone) during pupation. Wet season (WS) males have high levels of 20E and become active courters. Dry season (DS) males have lower levels of 20E and reduced courtship rates. However, WS courtship rates can be achieved if DS male pupae are injected with 20E at 30% of pupation. Here, we investigated the genes involved in male courtship plasticity and examined whether 20E plays an organizational role in the pupal brain that later influences the sexual behaviour of adults. We show that DS pupal brains have a sevenfold upregulation of the yellow gene relative to the WS brains, and that knocking out yellow leads to increased male courtship. We find that injecting 20E into DS pupa reduced yellow expression although not significantly. Our results show that yellow is a repressor of the neural circuitry for male courtship behaviour in *B. anynana*. 20E levels experienced during pupation could play an organizational role during pupal brain development by regulating yellow expression, however, other factors might also be involved. Our findings are in striking contrast to *Drosophila* where yellow is required for male courtship.

Visible and ultraviolet colours in an incipient species pair of *Colias* butterflies

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Mating cues evolve rapidly and can contribute to species formation and maintenance. The genetic architecture of such traits, as well as their genomic consequences in speciation, have recently become easier to study with technological advancements including genome sequencing and CRISPR-Cas9 mutagenesis methods. Two species of *Colias* butterflies, *C. eurytheme* and *C. philodice* came into secondary sympatry in the Eastern United States within the last century – both species have a variety of species-defining traits, but they also readily hybridise when they are at high frequency in agricultural sites. This has allowed us to map and characterize several genes responsible for between-species variation in colour which act as mating cues, including a transcription factor that controls the presence or absence of male-limited ultraviolet iridescence, and a novel pigment transport gene involved in regulating pterin pigments. It also offers a window into the population genomic consequences of secondary sympatry in an incipient species pair, including an outsized role for the sex chromosomes.

A complex colour polymorphism is associated with a gene duplication in wood tiger moths

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Colour is often used as an aposematic warning signal, with predator learning expected to lead to a single colour pattern within a population. However, there are many puzzling cases where aposematic signals are also polymorphic. The wood tiger moth, *Arctia plantaginis*, uses bright hindwing colours as a signal of unpalatability. Males have discrete colour morphs which vary in frequency geographically. In Finland, both white and yellow morphs can be found within the same populations, and these colour morphs also differ in behavioural and life-history traits which contribute to the maintenance of this polymorphism. In similar cases, such complex polymorphisms are controlled by supergenes. In contrast, we show that male colour is linked to a duplicated copy of a *yellow* family gene that is only present in the white morphs. This white-specific copy, which we name *valkea*, is highly upregulated during wing development, and we use CRISPR/Cas9 to knockout this gene to validate its function in producing white wings. The *yellow* family genes have been linked to melanin synthesis and behavioural traits in other insect species. Our results add to only a few examples of seemingly paradoxical and complex polymorphisms which are associated with single genes.

The genes behind the scenes of visual mate preference in *Bicyclus anynana*

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In most species, individuals select their mates based on specific preferred phenotypes or characteristics. Determining the genetic basis of these preferences is a key question in evolutionary biology. In the butterfly *Bicyclus anynana*, naïve females prefer males with 2 dorsal forewing eyespots (DFS) over males with 4 DFS. To identify the genetic basis of this innate mate preference, we conducted a series of experiments. First, we tested the heritability of preference for spot number by assessing the innate preference of offspring of females who mate preferentially with 4 DFS males. The offspring of these females had a significant preference for 4 DSF males, suggesting the preference is heritable. We then built a 4 DFS preference selection line by selecting for females that innately preferred 4 DSF in every even generation while the odd generations were allowed to breed freely and have maintained a female preference for 4 DSF males in this line for over 12 generations. To identify the genetic basis of this preference, we conducted a genome wide association study using WT females that preferred 4 DFS males and WT females that preferred 2 DFS males. We recovered a single large peak containing a small set of genes, suggesting that preference for spot number is controlled by a small number of genes of large effect. We are testing the effect of these candidate genes on mate preference using CRISPR Cas9 gene editing and mutant line development. Results from our microinjections and mutant line will be discussed.

A clinal polymorphism at the *optix* locus drives differences in silver scale iridescence in Mormon Fritillaries

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Many fritillary butterfly species have species-specific polymorphisms for the presence of iridescent silver scales on their ventral hindwings. In the Western US, the Mormon fritillary *Speyeria mormonia* have unsilvered morphs that are present at high frequency in northern populations, declining to less than 1% in Colorado, where silver morphs predominate. Our data show that the unsilvered trait is controlled by a dominant Mendelian locus. We identify the gene *optix* as responsible for the phenotypic switch and demonstrate that *optix* changes the nanoscale properties of scales, allowing the development of light-scattering iridescent ultrastructures. We also provide evidence that the *optix* locus has been under recent selection, and that divergent alleles may have introgressed from sympatric hybridising species. Our study sheds light on the genetic and functional basis of a clinal polymorphism, providing insight into the ecological relevance and evolutionary forces shaping the distribution of this trait.

Migration in butterflies: movement, physiology, interactions, and genes

KEYNOTE TALK

Migratory ecology and population dynamics of the Painted Lady butterfly, *Vanessa cardui*

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Butterfly migration is a natural phenomenon that has long fascinated scientists and naturalists, but our understanding of their population dynamics remains limited, mainly because of methodological challenges. Here, we show novel integrative approaches to reconstruct long-range butterfly movements, combining tools on genetics, isotope ecology, ecological niche modelling, pollen metabarcoding, remote sensing, field ecology, and citizen science. We will present the latest discoveries on the migrations of the Painted Lady butterfly (*Vanessa cardui*), the most cosmopolitan of all butterflies. First, we track the multigenerational movements of a migratory outbreak that took place in the Afro-Palearctic range in 2019. We use DNA metabarcoding to identify plants from pollen transported by butterflies in 14 countries over 7 months. Through combining species distributions models from identified plants, we trace back the location of the outbreak's origin in the Middle East and the origin of each of the subsequent generations across Europe and Africa. Second, we report a transatlantic crossing by *V. cardui* butterflies that spanned at least 4,200 km from West Africa to South America and that lasted no less than 6–8 days. This journey could expand to 7,000 km with a possible origin in Western Europe, being the longest documented for individual insects. Lastly, we will show how a model-based approach has been key to understand the monthly shifting distributions of *V. cardui* across its Afro-Palearctic range, transpiring across 15,000 km from tropical to boreal environments. In summary, our work contributes new methodological avenues to advance our understanding of the migration of butterflies.

A migratory divide for the Painted Lady butterfly (*Vanessa cardui*): A single genomic region differentiates populations in the two hemispheres

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The Painted Lady butterfly (*Vanessa cardui*) is an emerging model species for eco-evolutionary research of insect migration. Its annual migratory circuit between Europe and tropical Africa has been recently characterised, but movement dynamics in the Southern Hemisphere of Africa remain unexplored. We hypothesise that synchronous north-south migratory movements occur in each hemisphere due to an inverted seasonality regime, resulting in two populations that might rarely overlap, but that might inhabit the equatorial latitudes in the two different wet seasons. Here, we test whether these two independent migratory ranges exist in the Northern and Southern hemispheres by means of population genomics. We analyse ddRAD data for 300 individuals across Africa and Europe and find two genomic clusters generally matching the two hemispheres. Interestingly, genetic differentiation along the genomes is minimal, except for a single 9 Mb region showing high F_{ST} values. Such drastically differentiated region, coupled with its low intra-population diversity (π), is likely explained by a chromosomal inversion. This presumed rearrangement comprises at least 333 genes, of which the most divergent were enriched in relevant functions to migration. Our results will help to pinpoint candidate loci to understand the adaptive nature of migration, in this case, where two populations migrate in opposite directions as a response to similar cues. This study provides the first genomic evidence for a migratory divide in insects and highlights the importance of characterising the boundaries of populations for species that migrate over large geographical ranges.

Microbiota of adult butterflies – moving from spatial to temporal scales

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Host movement can have contrasting effects on the gut-microbiota. Aggregation could enhance inter-individual transmissions and uniformity among conspecifics at the same location (as seen in social or gregarious insects), while host dispersal or migration could dissolve location-specific effects. Though host movement and migration distance could directly influence timing of microbial acquisition it has been little regarded within host-microbiota frameworks. In contrast to the honeybee, which can rely on social exchange of microbes, solitary pollinators need to acquire their microbiota entirely from the environment. This makes solitary pollinator potentially more vulnerable to disturbances of microbial dispersal across plant-pollinator networks and environmental fluctuations that influence timing of microbial acquisition. In adult butterflies, recent studies point at the enrichment of an abundant and species poor community with important ecological function. But basic routes of microbial acquisition are still insufficiently understood in butterflies. A functional framework that integrates timepoint of microbial acquisition with dispersal abilities and movement range of the host will help to better understand principles of microbial community assembly for semi-migratory butterflies.

Danaid migration in Asia – revealing the mystery of movement using URL-branded tags

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During the dry season each year, thousands of migrating danaini form large, multispecies aggregations in Hong Kong. After a brief stay from November to January, they disappear – destinations unknown – until the following wet season. Danaini migration is understudied in Asia and as a result there is scant data on their movements within the Indopacific region. This lack of data limits our ability to identify flyways and enact appropriate conservation measures for migrating danaini and the ecological communities they inhabit.

To understand the seasonal movements of danaini aggregating within Hong Kong (1,104 km²), we began tagging butterflies with uniquely coded, bilingual tags branded with a URL at which members of the public can report sightings. Our use of publicly-reportable tags proved successful: in two seasons of data collection, we received nearly 250 public reports of 4,700+ tagged butterflies. These reports confirmed movement of *Euploea*, *Tirumala*, *Danaus*, and *Ideopsis* species <1 to 14 km (straight-line distance) over land and sea, between 1 and 132 days from their tagging date. Our mark-recapture data indicate that recent dry season aggregations consisted of approximately 20,000 individuals combined (95% CI: 15,000–28,000) – primarily *Euploea core* and *Euploea midamus*.

Our findings highlight the utility of citizen scientist reporting for migration and movement research. Given our successes thus far, we are expanding our tagging and outreach efforts to connect with citizen scientists across Asia. We need considerably more data on the migration dynamics of Asian danaini to inform even basic conservation measures for these poorly understood species.

Where does the swallowtail fly? – Results of a citizen science study at the Wurmlinger chapel in Rottenburg, Germany

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The swallowtail *Papilio machaon* is a well-known species especially because of its conspicuous hilltopping behaviour, flying to the summits of hills where they show perching and patrolling behaviour and find mates. Currently, it is not well understood how long *P. machaon* stays at these summits, how large the populations are, how far the butterflies disperse after leaving the mating site and where they fly.

The aim of this work was to investigate the dispersal and seasonal dynamics of a population of *P. machaon* at the Wurmlinger chapel north of Rottenburg. We combined three methods (1) CMR, (2) Citizen science using mass media reports (online, television, radio and print) to increase the recovery rate of marked *P. machaon* and (3) telemetry using Nanotags. Jolly-Seber models in MARK were used to estimate the population sizes.

A total of 153 swallowtails were caught and 65 recaptures were registered during 2022. The maximum age of *P. machaon* was at least 17 days. The population size of males was estimated to be 29 ± 5 in the first generation and 230 ± 34 in the second and third generation. Five observations from citizens were recorded with a distance of more than 1 km from the chapel. Using telemetry, we were able to record a dispersal distance of 2.5 km. To our knowledge, we were able to record the longest dispersal distance ever documented for *P. machaon*. A distance of 66 km was reached by a marked swallowtail, which was photographed by a citizen near Heilbronn.

Hydrogen and strontium isoscapes for the African Palearctic range to reconstruct insect migration and connectivity

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Many insects (e.g., *Vanessa cardui*) undertake multi-generational migrations across the Afro-Palearctic range. However, the migration routes and connectivity of these insects remain mysterious because insects are small, short-lived and have large populations. Hydrogen isotope values ($\delta^2\text{H}$) and strontium isotope ratios ($^{87}\text{Sr}/^{86}\text{Sr}$) are promising intrinsic markers to investigate migration and connectivity. However, a prerequisite to use multiple isotope geolocation for tracking insects is to build isotope baselines. Here, we present two novels $\delta^2\text{H}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ isoscapes for the Afro-Palearctic range. To develop a $\delta^2\text{H}$ isoscape, we collected resident butterflies across the range and measured the $\delta^2\text{H}$ of their wings. We then correlated this measured $\delta^2\text{H}$ with the $\delta^2\text{H}$ values from existing precipitation isoscape ($R^2=0.53$; $p\text{-value}=2.2\times 10^{-16}$). We used that relationship to predict the mean and standard deviation of $\delta^2\text{H}$ in butterfly wings across the Afro-Palearctic range. We explored the controls of the remaining model uncertainty to propose future directions to improve the modeling framework. To develop a $^{87}\text{Sr}/^{86}\text{Sr}$ isoscape, we collected plants from across the range and measured their $^{87}\text{Sr}/^{86}\text{Sr}$. We applied an automated spatial interpolation and prediction using Ensemble Machine Learning leveraging existing environmental geospatial data from the study area to predict the mean and standard deviation of bioavailable $^{87}\text{Sr}/^{86}\text{Sr}$ across the Afro-Palearctic ($R^2=0.73$; $\text{RMSE}=0.0032$). These new $\delta^2\text{H}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ isoscapes provide the framework to apply dual H-Sr isotope geolocation for insects across the Afro-Palearctic range. This framework brings a new approach to investigate the migratory connectivity of insect populations across the Afro-Palearctic with critical implications for conservation and biosecurity.

Isotope-based geographic assignment provides valuable insights into long-distance butterfly migration

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Studying the migratory patterns of butterflies is difficult because butterflies are too small, short-lived, and numerous to be efficiently tracked by traditional techniques, like radio-transmitters. However, endogenous biomarkers, such as isotopes, have become reliable tools for estimating the natal origins of migratory butterflies. Some isotopes vary predictably across the landscape, allowing for the creation of spatial models of isotope variability (i.e., isoscapes). By analyzing and comparing the isotopic signatures of a butterfly's wing to an isoscape, the natal origin of the butterfly can be estimated via isotope-based geographic assignment. Using the monarch butterfly (*Danaus plexippus* (L.)) as a case study, we show that combining hydrogen and strontium isotopes into a dual-isotope framework can increase the spatial precision of isotope-based geographic assignment, compared to using hydrogen alone. Applying this framework to the painted lady butterfly (*Vanessa cardui* (L.)) demonstrates trans-Saharan migrations from Europe to West Africa, and back, with individual butterflies travelling up to 3500 km. Interdisciplinary work combining this dual-isotope framework with population genomics indicates panmixia across the Sahara Desert, suggesting regular migrations in this area. By combining isotopes with metal composition, ecological niche modelling, pollen analysis, and wind-trajectory modelling, we can further refine our ability to estimate the natal origins of individual migratory butterflies. Overall, we highlight the advantages of combining hydrogen and strontium isotopes for the geographic assignment of migratory butterflies and the resultant advances to our understanding of butterfly migration.

Transcriptomic analysis reveals pathways underlying response to environmental stress in a migratory butterfly

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Migration is a complex behavior that involves the synchronization of a multitude of physiological and behavioral processes in response to environmental cues. In this study, we used the Painted Lady butterfly (*Vanessa cardui*) as a model and examined the gene expression profiles in response to stressors introduced both early in development (larval crowding and lack of food resources) and at the adult stage (host plant absence).

Our findings showed that both environmental stress treatments during early life stages resulted in a significant change in gene expression patterns. In particular, genes involved in development, reproduction, and metabolism were significantly enriched in response to crowding and starvation stress. Furthermore, in adult individuals raised in food limitation, the expression of genes involved in environmental sensing was altered, whereas, in response to crowding, immune genes were differentially activated.

Moreover, our examination of the adult response to host plant availability revealed significant modifications in gene regulation, particularly in the expression of ecdysone esterase and juvenile hormone. We hypothesized that the ecdysone pathway may play a crucial role in the rapid switch between dispersal and reproduction. In addition, our functional analysis of candidate genes revealed significant enrichment in lipid, carbohydrate, and vitamin biosynthesis pathways, as well as the metabolism of pathogens itself and expression of multiple immune genes.

Our study offers a comprehensive insight into the genetic basis of environmental response in migratory insects and highlights candidate genes which may underpin the migratory syndrome.

KEYNOTE TALK

Monarch butterfly migration: genomes, gene expression and parasites

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Monarch butterflies (*Danaus plexippus*) are known for their spectacular migration from eastern North America to Mexico, a journey that involves thousands of miles. Monarchs west of the Rocky Mountains migrate much shorter distances to reach their overwintering sites along the California coast. Because of these different migration strategies, it has often been suggested that eastern and western monarchs constitute different populations, but genetic evidence has been lacking. We sequenced the genomes of eastern and western monarchs and found no evidence of population structure. Assaying flight ability on tethered flight mills, and gene expression of candidate loci, we found that eastern monarchs fly more efficiently, and that they experienced lower gene expression related to nonmuscular motor activity. Migratory success is not only determined by monarch genomes, but also by infection with the protozoan parasite *Ophryocystis elektroscirrha*. Using a long-term data set, we found that parasite prevalence has increased drastically over the last decades. Parasites reduce monarch flight ability, and we estimate that tens of millions of monarchs are now unable to complete their migratory journey. Increased parasite prevalence is likely due to multiple reasons, including formation of sedentary monarch populations. Our results suggest that migratory differences in eastern and western monarchs are driven by differential gene expression, such that conservation efforts in eastern and western North America are beneficial for monarchs on both sides of the continental divide. Moreover, efforts to reduce further rises in parasite prevalence are important for monarch conservation.

Are Monarch Butterflies True Navigators: using Agent-based and Spatial Modeling to Explore Animal Navigation and Migration

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The annual migration of monarch butterflies is one of the most well-known animal movement behaviors. Individual monarchs, with a brain the size of a pin head, can travel upwards of 4,000 km from breeding locations in the continental United States and Canada to central Mexico. However, how this behavior is completed, i.e., the specific navigation strategy used by the animal, has been difficult to determine with commonly used behavioral assays. Specifically, it remains unknown whether monarchs are true navigators (i.e. they know their destination and adjust their direction toward it during the migratory journey) or vector navigators (i.e. they fly in a south-westerly direction regardless of the stage of their journey). True navigation is often characterized as the most accurate since the migrants know where their destination is. This accuracy often leads to the assumption that most large-scale migration must be the result of true navigation since the animals cannot rely on serendipity alone to complete such an important biological movement. Using the monarch butterfly as a case-study, my work tests this assumption using agent-based and spatially explicit modeling approaches. By simulating populations of agents, matching their behaviors to what is known about monarchs, I can compare how likely they are to reach the overwinter locations. This work helps answer whether migrants need to know where they are going to get there.

Butterfly communities: Evolutionary assembly, ecological dynamics, and multi-trophic interactions

KEYNOTE TALK

Ecological uniqueness of communities: controlling for environmental availability highlights importance of insecticide for urban butterflies

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Identifying the drivers that promote unique species compositions (i.e., ecological uniqueness) is crucial to understanding the mechanisms underpinning diversity patterns and for implementing effective conservation planning. Specifically, reduced ecological uniqueness reflects more similar species composition among sites, a pattern known as biotic homogenization. Environmental conditions are often sampled differentially in datasets, which can lead to rarer environments having unique species compositions, provided that environmental differences increase compositional differences. We developed an approach to reduce the effects of environmental availability in ecological uniqueness analyses through calculating expected pairwise compositional dissimilarities for each unique environmental condition. We applied this approach to a dataset of butterfly communities and environmental factors, including weather condition, vegetation structure and management practices, across urban parks in Hong Kong. Using traditional approaches to ecological uniqueness, we found that only plant cover had an effect on ecological uniqueness of the communities. However, using our new approach we found that only the frequency of insecticide spraying was important for uniqueness, suggesting that the plant effect is likely a sampling artefact. Our results demonstrate that insecticide spraying in urban parks can be a force contributing to biotic homogenization – a driver of change that remains poorly understood with respect to impacts on local diversity. Biotic homogenization caused by insecticide spraying can subsequently exacerbate diversity loss in urban areas at larger spatial scales. Furthermore, we highlight here the importance of considering environmental availability effects when assessing drivers of uniqueness in ecological communities.

The roles of parasitoids in butterfly communities

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For each butterfly species in a community there is a suite of parasitoid species. Some of these parasitoids are shared among butterfly host species and some are not. While adult butterflies aren't hosts to parasitoids, their eggs, larvae and pupae are. So for many species the majority of life is spent under threat of parasitism. Even hosts that are unpalatable or are defended against predation by other arthropods or birds, are still subject to parasitoids. Parasitoids are also common in most butterfly communities. Thus, since every time a parasitoid successfully reproduces, a host dies, parasitoids are directly associated with host fitness, are likely to drive aspects of evolution, and can impact the population and community dynamics of butterflies.

Using mostly examples from the relatively well studied parasitoids of European Melitaeini butterflies I will illustrate (1) the role of parasitoids in host population dynamics, which in a community context can be quite low, (2) how butterflies can influence one another through population sizes of shared parasitoids, and (3) parasitoid-butterfly co-evolution driving virulence and defense but not species diversification. Globally the identities and host ranges of most parasitoids are not known, nor are their rates of parasitism in any community. Some regions such as Western Europe, other select communities that have been studied using molecular methods, and parasitoids of agricultural or forest pests are exceptions that we know more about. But what we understand about the roles of parasitoids for ecology and evolution of butterflies in these specific contexts is not generalizable.

Nectar breadth and associations with population trends in Sierra Nevada butterflies

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Interactions with plants are an essential component of butterfly life histories, and specificity in larval host breadth has been implicated as a factor in population declines in various parts of the world. Diet breadth for adult life stages, has not been as widely studied, largely because butterflies are typically assumed to be nectar plant generalists. Recent work leveraging long-term monitoring data in the western United States has found a majority of species are in moderate to severe decline, with causes that include habitat loss, habitat degradation and climate change. Using these data in conjunction with our recently-collected data on adult diet breadth (“nectar breadth”) we are evaluating the following questions: (1) is there variation among butterfly species in nectar breadth?, and (2) does nectar breadth inform our understanding of butterfly population trajectories over time? During twice-monthly visits to five sites in the Sierra Nevada Mountains, each unique plant-butterfly association is photographed. Photographs are used to identify both butterflies and flowers to species, thus developing an understanding of nectar breadth among focal species. To increase sample size, we supplemented our database with observations from iNaturalist. Using rarefied estimates of nectar breadth, we do not detect a clear relationship between nectar breadth and long-term population trends, but early results indicate species with broader nectar breadth slightly decreased in abundance after a period of drought. We also found that not all butterflies are equally generalized, and we discuss the extent to which this axis of life history variation can inform conservation and monitoring.

Evolution of temporal niches in butterfly communities and consequences on sympatric speciation

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The co-existence of closely-related species in sympatry often implies the partitioning of species into different ecological niches. Whether this partition is the result of sympatric speciation or of a secondary evolution of traits within species in response to ecological interaction in sympatry is an open question. Here we focused on closely-related species of *Morpho* butterflies that occur in sympatry for a large part of their Amazonian range and display parallel variations in their iridescent blue wing colour patterns. Using experiments carried out in the field, we recently showed that their striking resemblance may lead to substantial sexual interactions between species. In this talk, I will present results from mark-recapture experiments documenting the temporal segregation in the timing of daily activities between these species, in different Amazonian localities. I will then investigate the selective pressures acting the evolution of temporal patterns of daily activities in sympatric species using a mathematical model. Finally, I will use numerical simulations to test for the effect of temporal segregation and genetic incompatibilities in the process of sympatric speciation. Altogether, this talk will highlight the key role of temporal pattern of reproductive activities in the assembly of species-rich communities of the Amazonian rainforest and show the key determinants for sympatric speciation caused by such temporal segregation.

Geography of butterflies of the Republic of India: Species richness and faunal composition patterns revealed via check-lists of federal states

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Butterflies are widely used to analyse biogeography patterns, both at the global level and in regional studies covering countries and continents. So far, most of the latter originated from well surveyed northern regions, while the species-rich tropical areas were lagging due to a lack of appropriate data. We used check-lists of 1379 butterfly species recorded in 36 federal states of the Republic of India (1) to assess the validity of basic macroecology rules, and (2) to relate species richness, distribution of endemics and geographic elements, and species composition, to geography, climate, land covers, and socioeconomic conditions of the states. Area, land covers diversity and latitude do not affect species richness of Indian federal states, whereas topographic diversity and precipitation/temperature ratio (i.e., available energy) display strong positive effects. We interpret this by geographic and climatic idiosyncrasies of Indian subcontinent, with the highest richness situated in small, densely forested mountainous northeastern states receiving summer monsoons. Peninsular effect, i.e., decrease of richness towards the tip of subcontinent, is counterbalanced by the mountainous rainforest region of the Western Ghats. Afrotropical elements are associated with savannahs and Palearctic elements with treeless habitats. The overwhelming effect of geography, linked to historical dispersal routes and barriers, is also reflected in species composition patterns. The bulk of Indian butterfly richness, and hence the highest conservation priorities, overlap with the global biodiversity hotspots, but the mountain states of the Western Himalayas and the savannah states of peninsular India also host distinctive faunas.

Brown and grey shades of African savannahs: Understanding the direct and indirect effects of the environment on moth communities in Kruger National Park

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Savannahs are a complex and dynamic ecosystems that are formed and influenced by multiple factors at different temporal and spatial scales, such as climate, weather, productivity, water availability, fire, and herbivory. These and other variables play an important role in determining the composition and structure of local and regional biodiversity. In this study, we examined the direct and indirect effects of large-scale variables (climate, environmental productivity) and local variables (water availability, disturbance by large herbivores, and vegetation structure, and composition) on moth communities in Kruger NP, South Africa. We applied structural equation models (SEMs) and multivariate ordination analyses to assess the relative importance of these factors using a dataset of 817 moth species systematically collected from 60 plots representing the regional variability of the savannah. We found that moth diversity was significantly affected by vegetation, climate, productivity, disturbance by herbivores, and water availability, in various combinations of direct and indirect effects. The relative contribution of these variables to moth community composition will also be presented. Our results revealed the complex interplay between the variables responsible for the formation of moth communities in savannah ecosystems. Such knowledge is crucial for our understanding of the dynamics of this important ecosystem, as well as for effectively conserving its biodiversity.

Seasonal shifts of biodiversity patterns and species' elevation ranges of butterflies and moths on Mount Cameroon

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Butterfly and moth communities in tropical mountains are vulnerable to climate change. However, their natural dynamics of seasonal shifts remain poorly studied, which strongly limits our understanding of their future changes. We quantitatively sampled nine groups of Lepidoptera (butterflies and moths) along the only complete forest elevational gradient in the Afrotropics on Mount Cameroon. Sampling was repeated over three seasons. Altogether, we recorded an impressive 42,936 specimens of 1,099 species. Our results show a mid-elevation peak of species richness for virtually all the studied groups. This peak shifted seasonally for five groups, with most of them ascending during the dry season. Seasonal shifts of species' elevational ranges were responsible for these diversity pattern shifts along elevation: we found general species' upward shifts in fruit-feeding butterflies, fruit-feeding moths, and Lymantriinae from beginning to end of the dry season. Conversely, Arctiinae shifted upwards during the wet season. Such shifts may lead to misinterpretations of diversity patterns along elevation if seasonality is ignored. We report seasonal uphill and downhill shifts of several lepidopteran groups, with average seasonal shifts of elevational ranges often exceeding 100 m and even several times higher for numerous species. Our findings suggest that natural temporal dynamics should be studied and taken into account when investigating the impact of global climate change on Lepidoptera communities in tropical mountains.

From top to bounds: Forest fragmentation emulates the vertical strata as a habitat filter for butterfly assemblages

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Understanding the patterns that emerge from habitat filtering processes, whether natural or human-induced, is a crucial step in designing strategies for biodiversity maintenance. In tropical forests, the vegetation structure provides distinct habitat conditions in vertical and horizontal dimensions, which consequently affects the species composition of local assemblages. We used two distinct datasets to evaluate the effect of forest fragmentation and vertical forest strata on the phylogenetic and functional diversity of Atlantic Forest butterfly assemblages. In addition, we tested the role of phylogeny on assembly patterns and morphological trait distribution in both scenarios. We hypothesized that the fragmentation process positively selects butterfly lineages more tolerant to disturbed habitats, which would be a similar habitat filtering imposed by forest canopy on the vertical dimension. We confirmed the distinct species and trait composition between continuous and fragmented areas and between forest understory and canopy, with vertical strata selecting specific lineages. In addition, we found convergence in weighted mean body size decreasing of butterfly assemblages from continuous to fragmented areas and from understory to the canopy. Phylogenetic and functional diversity presented an increasing trend toward forest understory, which highlights the importance of understory forested habitats for the maintenance of butterfly diversity. Our results demonstrated that forest fragmentation and vertical forest strata act under similar habitat filtering, selecting species and traits related to closed-forest species but in different spatial dimensions.

Evolutionary and ecological patterns of shelter-building across North American butterfly caterpillars

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Many caterpillars build shelters for protection from predators, environmental stressors, or other threats. Although shelters have not been consistently documented, they provide insights into ecological and evolutionary patterns. Shelters can be categorized based on construction materials and methods and include silk webs, various leaf shelters, and frass piers. As North American butterfly caterpillars' behaviors are well-described, we mapped caterpillar shelter information from published sources and iNaturalist onto a published phylogeny of North American butterflies to investigate the documentation of shelter-building and the shelters themselves. We asked whether there were patterns in published information about shelter-building and, for shelter-building caterpillars, we also asked whether evolutionary history, family, or geography were associated with different shelter types.

We found published shelter-building information for 452 species, with 73% of the records reporting shelter-building, suggesting a preference for recording shelter-building but not its absence. However, in many cases, there was no additional information about the shelter. This had some evolutionary correlation ($\lambda = 0.46$) and was more common if a species' range included the tropics ($p = 0.03$), emphasizing the importance of tropical studies. Leaf ties had little evolutionary signal ($\lambda = 0.096$), while tunneling into plants had a strong evolutionary signal ($\lambda = 0.57$) and was significantly less common in nymphalids compared to hesperiids ($p = 0.006$). This suggests that some types of shelter-building are much easier to adopt than others. In the future, we aim to extend this to both more butterflies, but also to moths, which contain the majority of shelter-building species.

Snow dominates dynamics of alpine *Parnassius smintheus* butterflies

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We use long-term population monitoring (27 years) of a 21-meadow network of populations, of alpine *Parnassius smintheus* butterflies, combined with experimental manipulation of winter conditions (snow) and satellite imagery of snow cover, to determine the role of over-winter egg survival on population dynamics in the Rocky Mountains of Alberta, Canada. Over-winter survival of experimentally planted eggs explained 80–90% of the variation in annual population change (R_t) of adult butterfly abundance between consecutive summers. Experimental removal of snow cover resulted in very low, or no, survival of eggs in each year. These patterns support results from long-term population monitoring of *P. smintheus*, wherein the interaction between winter temperatures and snow cover are the best predictors of population change.

P. smintheus oviposit near, but not on its hostplant, *Sedum lanceolatum*. Oviposition site-choice by females in mid-summer, is clearly done without knowledge of snow cover the following winter. Using high-resolution (30 cm) satellite imagery of snow accumulation, and systematic mapping of hostplant distribution, we find a strong spatial association between patterns of snow cover in winter and of *Sedum* abundance. *Sedum* is more abundant where snow accumulates. Snow cover is therefore not only associated with abundant larval resources, but also with high egg survival; its variation thus dominating *P. smintheus* dynamics. Results suggest that loss of alpine snow cover, due to climate change, may place these butterfly populations at risk.

Dispersal barriers in Alpine populations of Copper butterflies

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Dispersal is a key biological process serving several functions including connectivity among populations. Investigating factors affecting dispersal, gene flow, and genetic diversity is important in the current era of anthropogenic global change, as they have implications for a species' resilience to environmental change. Using landscape genetics / genomics, we investigated gene flow and genetic structure in three species of Copper butterflies (*Lycaena* spp.) in the European Alps. In *L. tityrus*, we found surprisingly high levels of gene flow at the local level. Nevertheless, ravines, forests, and roads had effects on genetic structure. At larger scales, we found strong genetic differentiation across the Alps with four genetic clusters, indicating different glacial refuges, and a strong reduction of genetic diversity from west to east. In the other species, we found a higher genetic diversity and a more pronounced genetic structure in *L. hippothoe* than in *L. virgaureae*. Both species displayed a major genetic barrier in the central Alps, and were structured by minimum and maximum altitude. Our studies revealed (1) several structures affecting gene flow in Copper butterflies, (2) areas harbouring particularly high or low genetic diversity, and (3) interesting differences even between closely related species. These findings in turn have important implications for conservation. For instance, we suggest efforts to conserve Alpine Copper populations should focus on (1) the genetically diverse populations in the western Alps in *L. tityrus*, (2) connecting suitable habitats in *L. hippothoe*, and (3) increasing local population sizes in *L. virgaureae*.

Phenotypic plasticity in butterflies: from traits to their mechanisms and everything in between

KEYNOTE TALK

Pupal colour plasticity in tropical butterflies

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Pupal colour of many butterflies is a plastic phenotype, being either green or brown. In the satyrine *Mycalesis mineus*, we show that pupae on leaves are exclusively green, although green pupae are also found on off-leaf substrates. Brown pupae are found exclusively off-leaf. This correlation between pupal colour and substrate may help pupae avoid detection by predators. Brown pupae are more likely to form when larvae grow in arid conditions, when the surrounding foliage is brown, and this may enhance crypsis. High larval density induces a greater frequency of brown pupae, and I discuss how this can benefit the butterfly. In a comparative experiment involving 5 satyrines, we show that species with only green pupae tend to pupate exclusively on leaves, while pupae with only brown pupae tend to pupate off-leaf. Species with both green and brown pupae pupate both on leaf and off-leaf substrates, with leaf-borne pupae being green. Our experiments show that the pierid, *Catopsilia pomona*, which has pupae varying continuously from green to brown, is able to sense fine-scale variations in substrate colour, and match its pupal colour. The results from these studies support the idea that pupal plasticity is an anti-predator strategy. Off-leaf pupae are also likely to be under greater desiccation stress. We hypothesized that brown pupae are more desiccation tolerant than green ones. In another pierid, *Eurema blanda*, we show that brown pupae lose lesser water and have a higher eclosion success under desiccation stress, supporting our hypothesis.

Flexible transcriptional response to host plant switches in the comma butterfly (*Polygonia c-album*)

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Understanding the dynamics of interspecific interactions between insects and plants is one of the central topics in biological research. However, despite their ecological and economical relevance, very little is known about the actual mechanisms underlying these associations. While specialists only use a very limited set of plants, generalist species encounter a broader range of morphologically and chemically diverse hosts. This diversity can represent a challenge since it requires a flexible set of adaptations to the different host plants within a repertoire. Previous studies already showed that the use of a particular plant is associated with a specific gene expression profile. Host switch experiments were now combined with RNA-sequencing to further investigate these patterns in the polyphagous comma butterfly (*Polygonia c-album*). It could be shown that larvae not only differed in their response to a host, but could also quickly adjust their transcriptional profiles when translocated to a new plant. Some switches, however, seemed to be more challenging than others, indicating a potential involvement of trade-offs. The high flexibility suggests that besides host-specific gene expression also the ability to switch between these profiles is essential for the use of a broad host range. In an evolutionary context, this can provide important insights into the basis of host range expansion and phylogenetic recurrence. Given the resemblance between insect-plant and parasite-host interactions, these findings will also significantly increase our knowledge and the predictability of other species interactions.

Developmental plasticity in multifactorial environments: temperature and food quantity effects on *Bicyclus anynana* wing patterns

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Butterflies are valuable models for integrative studies of developmental plasticity. This property, which refers to the fact that phenotype depends on the environmental conditions experienced during development, can help organisms cope with environmental heterogeneity, such as that characteristic of alternating yearly seasons. Experimental studies of developmental plasticity often look at effects of single environmental factors. However, natural environments are much more complex, varying for multiple environmental factors whose combined effects on phenotype might not be additive. The African tropical satyrid butterfly *Bicyclus anynana* (Butler, 1879) is a species that exhibits seasonal polyphenism in habitats with alternating dry and wet seasons that differ in humidity and temperature, as well as in quality and availability of food plants. The seasonal morphs differ in wing pigmentation associated with distinct seasonal strategies to escape predators. During laboratory rearing, seasonal phenotypes can be induced by lower and higher developmental temperatures, respectively. While we know about the phenotypic effects of temperature and their physiological underpinnings, we know little about interactions with other environmental cues that also vary seasonally. We investigated the effects of temperature in combination with food quantity on adult wing pigmentation in different *B. anynana* families. We found significant effects of food quantity and significant non-additive interactions between food and temperature, with plastic responses to food quantity being stronger at higher temperatures. We also found genetic variation for thermal plasticity, but not for food-induced plasticity.

Variation in developmental plasticity in a temperate butterfly across a latitudinal cline: connecting phenotype and transcriptome

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Phenotypic plasticity may play a crucial role in the persistence of species under climate change as it allows for a more rapid response to environmental stress than through evolutionary adaptation. However, sensitivity to temperature may differ between traits, populations or individuals, and inferring general patterns is complex. Here, we used the Glanville fritillary butterfly (*Melitaea cinxia*) larvae originating from a latitudinal cline across its European range to assess whether plasticity in response to temperature varies with latitude (i.e. are there gene x environment (GxE) interactions) and whether potential mechanisms underlying phenotypic patterns can be inferred by differences in gene expression.

We performed a full-factorial common garden experiment where we measured development time, mass and growth rate in pre-diapause larvae from Finland, Estonia, Belgium and Spain that were reared at four temperatures (25, 28, 31 and 34 °C). RNA samples were taken from Finnish and Spanish larvae at the two extreme thermal treatments.

Our results show clear differences in thermal plasticity across the latitudinal gradient. Spanish larvae show a more canalized response for high mass at diapause across all temperatures whereas the Finnish larvae are canalized for a fast pre-diapause development time. This suggests intraspecific variability in the trade-off between mass and development time. Preliminary analysis suggests clear differences in gene expression between population and temperatures with more complex patterns in GxE expression.

In this talk, I will provide insights into how the environment differently affects populations across a latitudinal gradient and how this is mediated by changes in gene expression.

Local adaptation vs. phenotypic plasticity in traits associated with altitudinal adaptation in *Heliconius* butterflies

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Elevation, the vertical distance from sea-level to the top of the highest mountains, causes major and consistent changes in three climatic features: ambient temperature, atmospheric pressure and solar radiation. This climatic variation, in turn, is often associated with phenotypic variation along altitudinal gradients. Depending on the trait of interest, its altitudinal variation can be the outcome of local (genetic) adaptation through natural selection or a plastic response to the varying environment. In many cases, the observed phenotypic variation can be an interplay between some degree of adaptive genetic divergence between populations coupled with some adaptive phenotypic plasticity. A full understanding of the nature of phenotypic variation along altitudinal gradients is not only indispensable to understand the evolutionary processes of adaptation, speciation and diversification, but also to learn how living organisms may cope with long-term climatic changes (such as global warming). We performed a reciprocal transplant experiment between populations of two well-studied species of *Heliconius* butterflies (*H. erato* and *H. melpomene*), along an altitudinal gradient of the Andes in Ecuador, to assess the relative roles of local (genetic) adaptation vs. phenotypic plasticity in determining the altitudinal variation in morphological, life history, and thermal-tolerance traits. We found that certain morphological traits are less plastic than life history or physiological traits while the relative importance of adaptive population divergence and phenotypic plasticity also varies between the studied species. The next step will be to look at actual DNA-sequence changes associated with population divergence and gene expression changes associated with plastic responses.

Dynamic gene expression during a social learning event in a butterfly

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In many animals, individuals can learn to change their mating preferences after exposure to a new phenotype. In the butterfly *Bicyclus anynana*, both sexes learn visual preferences for wing patterns after three hours of exposure to a new phenotype. This learned preference is associated with changes in gene expression, with both fast and slow responses to stimuli. Therefore, genes critical to learning may be missed if we only examine gene expression at the time point when learning has been observed (3hrs). To identify the genes associated with learning across time, we conducted a RNA-Seq time series by exposing females to an unfamiliar male phenotype for different exposure periods, from 30 minutes to three hours, and pairing those with naïve females isolated for the same amount of time. We also conducted a learning time series by exposing females to an unfamiliar male phenotype for the same exposure periods and recorded their choice between the familiar and unfamiliar males after two days. By comparing the transcriptomic profiles between trained and naïve females across time, we found a peak of differentially expressed genes (DEGs) after one hour of exposure. Of these DEGs, 40 are putatively associated with learning and memory. We also found a peak of learning after three hours of exposure. Interestingly, the learning curve generally correlated with gene expression pattern, emulating a “two-wave” model. Our results show that both gene expression and behaviour are dynamic, with peaks at different time points. We also elucidate possible genes conserved during the learning process.

The neurogenomics of diversity in butterfly mate preference learning

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Mate preference learning, where individuals learn to prefer or avoid phenotypes based on social experience, is pervasive across animal taxa. However, not all animals learn preferences for the same thing, whether in terms of trait learned, valence associated with said trait, or salient cue used to induce a behavioral change. To uncover the neurogenomics underlying this variation in learning, we utilized two butterfly species that exhibit preference learning variation: *Bicyclus anynana* and *Heliconius melpomene*. Male and female *B. anynana* both learn positive visual preferences, but learn preferences for different traits; while different lineages of *H. melpomene* exhibit differing degrees of aversive courtship learning. To identify genes associated with this diversity in learning, we examined gene expression profiles of the brains and eyes of male and female *B. anynana*, and of the brains, eyes, and antennae of male *H. melpomene malleti* and *H. m. rosina*, immediately post training and compared them to the same tissues of naïve individuals. In *B. anynana*, we found more differentially expressed genes in the eyes, suggesting a role of the peripheral nervous system in imprinting-like learning. Furthermore, chemosensory genes, and genes that influence wing pattern, such as *spalt* and *invected*, were also differentially expressed. In *H. melpomene*, we found more differentially expressed genes in the brains, suggesting that the strength of learned response may be driven by higher processing. Our results suggest that different suites of genes and gene networks are associated with diversity in what versus whether animals socially learn.

Connecting plasticity across life stages

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Most organisms display phenotypic plasticity in a diverse range of traits. Simultaneous changes in different traits are often integrated, as seen in many seasonal polyphenisms, which can involve coordinated changes in traits ranging from wing color and morphology to life history strategy. But what happens when these plastic changes occur at different times in the life cycle, and what connects these plastic changes? To address these questions, we studied two forms of seasonal plasticity in *Pieris napi* butterflies. Facultative pupal diapause in *P. napi* is well studied, but the species also has poorly studied seasonal plasticity in adult wing coloration. We revisited our previous common garden study on urban evolution of seasonal plasticity in *P. napi* to show that pupal development pathway and adult wing color are tightly linked. But what connects these traits? Two possible explanations are: (1) developmental integration persisting across life-stages, or (2) a cue-mediated interaction where diapause alters the environmental cue for wing color. Specifically, pupal development pathway determines the temperatures experienced as a pupa, both in the wild and in our previous study, and if pupal temperature is a cue for wing color, it would connect these traits even in the absence of a physiological connection. We are currently testing this possibility by manipulating the temperature experienced by pupae independently from diapause pathway. Understanding the connection between plasticity in pupal development pathway and wing coloration will, in turn, help understand how integrated their evolution should be, including as a response to anthropogenic changes in seasonality.

Genetic constraints in genes exhibiting splicing plasticity in facultative diapause

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Phenotypic plasticity is produced and maintained by processes regulating the transcriptome. While differential gene expression is among the most important of these processes, relatively little is known about other sources of transcriptional variation. Previous work suggests that alternative splicing plays an extensive and functionally unique role in transcriptional plasticity, though plastically spliced genes may be more constrained than the remainder of expressed genes. In this study, we explore the relationship between expression and splicing plasticity, along with the genetic diversity in those genes, in an ecologically consequential polyphenism: facultative diapause. We compare these sources of transcriptional plasticity between diapausing and direct developing pupae of the butterfly *Pieris napi*. Splicing differs strongly between diapausing and direct developing trajectories but alters a smaller and functionally unique set of genes compared to differential expression. We further test the hypothesis that among these expressed loci, plastically spliced genes are likely to experience the strongest purifying selection to maintain seasonally plastic phenotypes. Genes with unique transcriptional changes through diapause consistently had the lowest nucleotide diversity, and this effect was consistently stronger among genes that were differentially spliced compared to those with just differential expression through diapause. Our results suggest that maintenance of the molecular mechanisms involved in diapause progression, including post-transcriptional modifications, are highly conserved and likely to experience genetic constraints, especially in northern populations of *P. napi*.

Hox gene *Antennapedia* potentially regulates butterfly eyespot size plasticity

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Many tropical butterflies evolved the ability to change the size of their eyespot patterns on the wing in response to alternating seasons. In African *Bicyclus anynana* butterflies, ventral hindwings eyespots are larger in the hot (27 °C) wet season form (WS), compared with those in the cold (17 °C) dry season form (DS) of the butterfly. This eyespot size plasticity helps them evade different predators present in each season. While previous studies suggest that this eyespot size plasticity, driven by temperature, is associated with different profiles of an insect hormone, 20E, little is known about the essential genes involved in the regulation of this plasticity on the wing itself. Using spatial transcriptomes generated by laser microdissections, we identified a Hox gene, *Antennapedia* (*Antp*), as the earliest temperature-sensitive positive regulator of eyespot size in *B. anynana*. Higher temperature induces a larger expression domain of Antp protein in the eyespot centers in the WS form, compared with the DS form. This differential expression is observed before the 20E sensitive stage, thus it is likely independent of 20E signaling. Knocking-out *Antp* reduces the size of the hindwing eyespots in the WS form, mimicking the DS form. From a phylogenetic perspective, only the *Satyrinae* butterfly subfamily (including *B. anynana*) display both the temperature-mediated eyespot size plasticity and the expression of *Antp* in eyespots. This suggest that the co-option of *Antp* into the eyespots of satyrid butterflies might contribute to the evolution of temperature-mediated eyespot size plasticity in this clade.

Is it hot in here? How thermal stress drives life-history variation, and the physiological mechanisms of this, in *Cinxia* butterflies

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Throughout their lifetimes, individuals must differently invest resources into distinct biological processes to maximise fitness outcomes at any one timepoint. Resource allocation trade-offs underpin life-history theory and lead to multiple pathways of conspecific variation in natural populations. However, how the dynamics and outcomes of these trade-offs change in response to unstable environments is not well known across taxa. Moreover, our understanding of how physiological mechanisms may mediate such trade-offs, especially in variable environments, is also comparatively lacking. Using a lab-reared butterfly population (*Melitaea cinxia*) as part of a common garden experiment, we investigated how males responded to three different nocturnal night treatments (control 8°C, alternating night-to-night 18° / 8°C, consistently warmer 18°C) in a number of life-history traits. We then assessed resting metabolic rate (RMR) as a potential trigger for re-investment into other life-history processes in the face of increased environmental stress. Finally, we outline whether any parental stress effects impact or benefit offspring that endure the same stressors during their development by rearing an F1 lab generation. Our results imply parent / offspring conflicts in responses to different thermal stresses, and differences in the costs of life-history across different developmental stages. Consequently, our results contribute to a more informed understanding of how climatic instability may shape evolutionary theory and give an insight as to how life-history trade-offs operate under different environmental stress contexts.

Covariation in wing color, behavior, and sensory gene expression in a seasonally plastic butterfly

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Many butterfly species exhibit seasonal polyphenisms in wing pattern. Behavior and physiology can also change with time of year, but in most butterfly species it is unclear if these seasonal behavioral and physiological phenotypes covary with wing pattern. To determine if wing color, behavior, and vision covary throughout the season in natural settings, we surveyed the common buckeye *Junonia coenia*, examining if its shift in summer to fall coloration is accompanied by a similar shift in behavior and gene expression, particularly in phototransduction and clock genes. We assayed behavior in 2018–2020 every other week from late May through October during focal watches and point counts in prairies in Arkansas, USA. On alternate weeks we collected animals for wing scores and in the early summer and fall collected animals for RNA-Seq analysis of eye tissue. Animals exhibited seasonal patterns of behavior in both point counts and focal watches, with the proportion of animals observed basking and the proportion of time spent basking increasing as temperature decreased in September and October. This change in behavior coincided with the shift towards darker “fall-like” wing colors in September. RNA-Seq analysis showed no seasonal difference between expression of opsin genes, which are responsible for color discrimination. However, we found that fall animals expressed higher levels of the gene *period*, which is responsible for regulating circadian rhythm and activity levels. Our study uncovers that seasonal changes in wing color are accompanied by increased thermoregulatory behavior and changes in clock gene expression in an abundant grassland butterfly.

Seasonal plasticity in the African butterfly *Bicyclus anynana* is facilitated by substantial shifts in regulatory molecular mechanisms

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Phenotypic plasticity is widespread in butterflies and has facilitated their adapt to seasonally fluctuating environments. The African butterfly *Bicyclus anynana* produces distinct wet and dry season forms that differ extensively in wing pattern, life history and behavioural phenotypes. To understand how plasticity evolves, and whether seasonally plastic species can adapt to future climate change, we need to understand the molecular mechanisms underpinning such processes. Here we explore the regulatory architecture of seasonal plasticity in life history strategies *Bicyclus anynana* using whole transcriptome data from abdomen tissue. We characterised variation in cis-acting regulatory variants between females reared under wet and dry season temperature treatments and found that 2,823 genes (representing 19% of genes across the genome) showed divergent cis-regulation between the wet and dry season treatments. We further show that 30% of genes under divergent cis-regulation are associated with genes that show significant differential expression patterns across GxE reaction norms. Interestingly, we found that 30% of genes under divergent cis-regulation genes showed purely plastic (i.e., environment only response) shifts in expression, which was more than expected by chance. Together, these results suggest a major role for cis-regulatory mechanisms in facilitating rapid adaptive shifts in life history phenotypes to seasonal environments. Moreover, our results evidence at least partial heritable basis to seasonal plasticity upon which selection can act, highlighting a potential adaptive capacity to future climate change.

Unravelling the Molecular Mechanisms of Adaptative Seasonal Plasticity in *Bicyclus anynana* Using Co-Expression Networks

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Adaptive seasonal plasticity is a prevalent phenomenon in organisms where individuals alter their phenotype in response to seasonal environmental changes. *Bicyclus* butterflies have a well-defined phylogeny and are an ideal model organism for studying adaptive seasonal plasticity due to their ecologically relevant seasonal forms. *B. anynana*, a member of this genus, has distinct wet and dry season forms with plastic traits such as wing pattern, reproduction, and behaviour, making it an excellent candidate for studying the molecular mechanisms underlying adaptive seasonal plasticity.

These complex traits often arise from the interaction between various genes and their molecular regulators, forming a dynamic network that can rewire in response to changing environmental conditions. The principal objective of this study is to investigate the rewiring of gene networks involved in adaptive seasonal plasticity. To achieve this, we employed differential co-expression analysis to explore the co-expression networks of genes expressed in the thorax and abdomen tissues of *B. anynana*'s wet and dry season forms. This robust tool offers a direct depiction of gene interactions, enabling a better understanding of how gene networks are rewired.

We hypothesize that the co-expression networks of genes expressed in thorax and abdomen tissues are rewired between different seasons, involving changes in network and module preservation, connectivity, centrality, or absence of certain genes in the co-expression networks, or variation in the co-expression values. Our study results will provide insights into the molecular mechanisms underlying seasonal adaptation in *B. anynana* and contribute to a better understanding of adaptive seasonal plasticity in organisms.

Are plastic responses to temperature, humidity, and host plant mediated by larval growth in the seasonally polyphenic butterfly *Melanitis leda*?

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Butterflies often show adaptive phenotypic plasticity where individuals use environmental cues during early stages to produce an adult phenotype that maximizes their fitness in the environment. In tropical satyrines, low temperatures and poor food quality experienced by larvae can induce the development of dry-season-form adults. Since both these factors reduce growth rate, larval growth may be part of the pathway that translates the environmental cues into the expression of phenotypes. To test this hypothesis for the common evening brown butterfly (*Melanitis leda*), we measured effects of temperature, humidity, and host-plant species on both larval growth and adult phenotype. We predicted that if larval growth rate is mediating the plastic responses, slower growing larvae would be more likely to develop a dry-season phenotype not only among treatments, but also within treatments. We found that even though among treatments larvae with lower growth rates were more likely to develop dry-season phenotypes (low temperature, low humidity, particular grasses), within treatments, growth rate was not linked to seasonal form of the adult. Therefore, in this species, plastic responses to environmental cues are not mediated by larval growth. This indicates that mechanisms of seasonal polyphenism fundamentally differ between clades of satyrine butterflies.

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KEYNOTE TALK

Long Distance Migration as a Driver of Sensory Plasticity and Evolution in Monarch Butterflies

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Migration over continental scales exposes individuals to disparate, temporally variable environments across their lifetimes. Naïve migrants, those on their first or only migratory trip, regularly encounter novel environments where they must successfully navigate, forage, reproduce, or socialize. As a result, sensory systems in long-distance migrants, and in naïve migrants in particular, should differ from non-migrants whose experience is more spatiotemporally constant. We use life history variation among monarch butterfly (*Danaus plexippus*) populations to explore how sensory capability varies as a result of long-distance migration. Behavioral (two-choice) assays using a nectaring resource reveals the predominance of visual cues for foraging. Initial preference suggests that monarchs possess an innate foraging preference. Innate foraging preference is seasonally-plastic and requires multi-modal sensory integration. Using imaging-based assessments of neuroplasticity, we provide initial suggestions for neural correlates of sensory variation among populations. Altogether, migratory monarchs appear to have evolved seasonally-plastic sensory differences that may allow for more efficient foraging in novel environments along their North American migratory route.

Butterfly monitoring, trends and indicators: towards a global network

KEYNOTE TALK

Envisioning the Indian Butterfly Monitoring Scheme: Harnessing the Power of a Billion Citizen Scientists for Tropical Biodiversity Monitoring and Conservation

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Biodiversity exploration and conservation are at critical crossroads. While there is renewed interest in discovering new species and documenting local and global biodiversity, the threats of population/species extinction have also become more acute because of human activity. Progress in biodiversity sciences and conservation is benefitting from large citizen science projects. Here we describe long-term monitoring under the Indian Butterfly Monitoring Scheme (iBMS), in the world's largest democracy that has stewardship of four globally significant biodiversity hotspots. The scientific goal is to record long-term trends in butterfly population dynamics in the face of changing climate, habitats, and land-use practices. Short-term butterfly monitoring efforts started in India in 1992, and long-term monitoring in 2008, with the longest (and still ongoing) time-series being 11 years with an interval of two weeks between successive counts. This systematic, periodic population monitoring is supplemented by opportunistic sampling of spatio-temporal occurrence through several citizen science platforms. These methods together have generated approx. 600,000 data points of 1,100 butterfly species, including records of many endemic and legally protected species. These developments have occurred at an opportune time: broad citizen engagement through social media, mobile apps and multi-platform aids in species identification and data recording have revolutionised biodiversity sciences, at the same time as India's scientific output is soaring. iBMS should serve as a model for other biodiverse countries as well as for integrating scientific efforts across long-term butterfly monitoring schemes to create a truly paradigm-shifting understanding of butterfly persistence through the world's space and time.

Butterfly monitoring in southern Mexico: challenges and prospects

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Butterflies can be suitable biological indicators as they are well documented, measurable, sensitive to environmental change, and are popular with the public because of their attractiveness. Study cases from narrowly-distributed and endemic butterflies are presented here as examples of insect monitoring in southern Mexico. The cases involve approaches for long term monitoring and auto-ecology, the assessment of climate change on mountain cloud forest species, with a focus on single taxa, or a subset of species in functional groups. Organized efforts have allowed the monitoring of key insect groups as a representative indicator of other animal groups from fragile ecosystems in Mexico. A challenge for insect monitoring and conservation in Mexico is building capacity to cover threatened taxa and monitor species occupancy and (ideally) abundance across key habitats throughout rapidly changing landscapes. Within this framework, other highly-diverse insect groups must be included in the national conservation agenda. Development of a robust monitoring scheme may further require an openness to emerging technologies (e.g. image and acoustic sensors, molecular methods) and different knowledge domains (i.e. state authorities, non-governmental organizations, research institutions, biological field stations, private sector, and citizen scientists/general public). This information will feed the currently poor databases where insect occurrence data are documented.

Tracking butterfly distributions over 200 years in American north of Mexico

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Insect declines are being observed globally but linking the multiple stressors and their interactions to specific changes in biodiversity patterns is difficult because dominant drivers continually shift in type and intensity over time and space. While public monitoring of butterflies has accelerated rapidly, most formal monitoring programs have been launched only in the past 20 years. To understand how butterfly distributions have changed over a longer history of intensifying human activity, we must link contemporary monitoring to historical data sources. Museum collections provide a wealth of distribution information, but only about 10% of North American butterfly specimens have been digitized. Further, focusing only on collections ignores the on-the-ground observations of amateur and professional naturalists, which have been published in the natural history literature and formal species accounts for centuries. These resources have been largely untapped in North America and we have undertaken an effort to compile a history of butterfly observations and link them with contemporary monitoring data to build a new comprehensive data resource. I present a 200 year time-series for two species: the regal fritillary (*Argynnis idalia*), a declining grassland species and the checkered white (*Pontia protodice*) which was quickly replaced by the European cabbage white (*Pieris rapae*) after its introduction in 1860. I will also present our plans for developing models that integrate naturalist observations, modern monitoring program data, species life history traits, and long-term land cover and climate data layers to better understand the multiple, interacting drivers of butterfly declines in America north of Mexico.

Butterfly monitoring in Peninsular Malaysia: Experiences, challenges and the way forward

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Peninsular Malaysia is home to more than 1,000 butterfly species. Despite its species richness, it is only in the beginnings of work on monitoring butterflies. Several short-term monitoring programmes have been conducted under various research projects since the 1990s. The earliest initiative focussed on the Swamp Tiger (*Danaus affinis malayanus*) in a nature park from 1998–1999, using a modified Pollard walk. A more recent monitoring programme was conducted for the Rajah Brooke's Birdwing (*Trogonoptera brookiana albescens*) from 2009 to 2014 using three different methods in two different study sites. A new monitoring protocol was developed based on counts of the puddling birdwings. Other ecological projects have incorporated habitat-level monitoring of species richness and abundance in ecological corridors and adjacent forests, recovering logged-over forest, and forest at different elevations. These projects utilized methods and sampling plots that may not be easily adapted for long term monitoring. Nevertheless, species- and generic-level abundance data from these projects could provide some baseline data for future monitoring programmes. The suitability of existing monitoring methods for tropical forests is discussed. There is a need for a greater range of methods that take into consideration the numerous tropical habitats and the behaviour of many elusive species. The major challenges in implementing long term monitoring in Malaysia are sustainable funding and adequate human resources. Moving forward, there is a need to incorporate monitoring as a regular component of larger ecological projects that attract funding and utilize the potential of volunteers in the implementation of long-term monitoring.

The Nigeria Butterfly Network: Towards the establishment of a nationwide Nigerian butterfly monitoring programme

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The Nigerian butterfly fauna is incredibly rich: over 1,300 species are known from the country. This diversity is due to several biogeographic zones meeting within the country, and the presence of multiple endemic species, predominately in isolated highland areas. However, as Africa's most populous country, Nigeria is threatened by serious biodiversity loss, both by its fast-growing economy and population. Currently, there are only few Nigerian environmental monitoring projects, and this hinders assessments of habitat quality and impacts from anthropogenic factors.

Long-term data from standardised monitoring of European butterflies have proven invaluable for conservation decision-making, and the detection of phenology shifts linked to climate change. However, employing the same methods in sub-Saharan Africa without prior validation is unlikely to return reliable data due to the markedly different faunal composition and habitat diversity. The different socio-economic situation also means that strategies to effectively recruit volunteers need careful consideration. Suitable field guides for beginners taking an interest in butterfly monitoring are also lacking.

The newly created Nigerian Butterfly Network aims to bridge these gaps through the following projects. Firstly, a new PhD project will perform a large-scale comparison of potential monitoring methods. Secondly, digital identification material, suitable for use by amateurs, is being produced and distributed for free. Thirdly, social surveys and outreach events will identify and support key groups most likely to be interested in volunteering for monitoring work. Together, these projects will help set the scene for a locally-informed nationwide monitoring protocol for Nigerian butterflies through the participation of citizen scientists.

The Israeli Butterfly Monitoring Scheme (BMS-IL): what happens to species at the desert's edge?

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Israel is located at the meeting point between Africa and Asia, and is characterized by a sharp precipitation gradient (from >1000 to 25 mm) and a diverse topography (-430 to +2200 m asl). Accordingly, Israel's butterfly fauna is highly diverse, including both Eurasian species reaching their southern distribution edge, and African (including Saharan) species reaching their northern edge. With rapid land-use changes, Israel offers a unique opportunity to study the impacts of both climate and land-use changes on ecosystems.

Established in 2009, the Israeli Butterfly Monitoring Scheme (BMS-IL) has by now ~190 active transects, mostly in the Mediterranean parts of the country. These are organized around a unique community-based structure. Based on the rich dataset collected by volunteers, we analysed how butterfly occurrence and abundance are affected by temperature and rainfall. We found that temperature affected occurrence, while rainfall affected abundance, such that most species will be negatively affected by climate change. We also assessed the status and trends of all species using the IUCN "Red book" criteria. We found that 46 out of Israel's 140 native butterfly species (32.8%) of the species are threatened according to IUCN criteria. The most dominant risk factors are climate- and land-use changes.

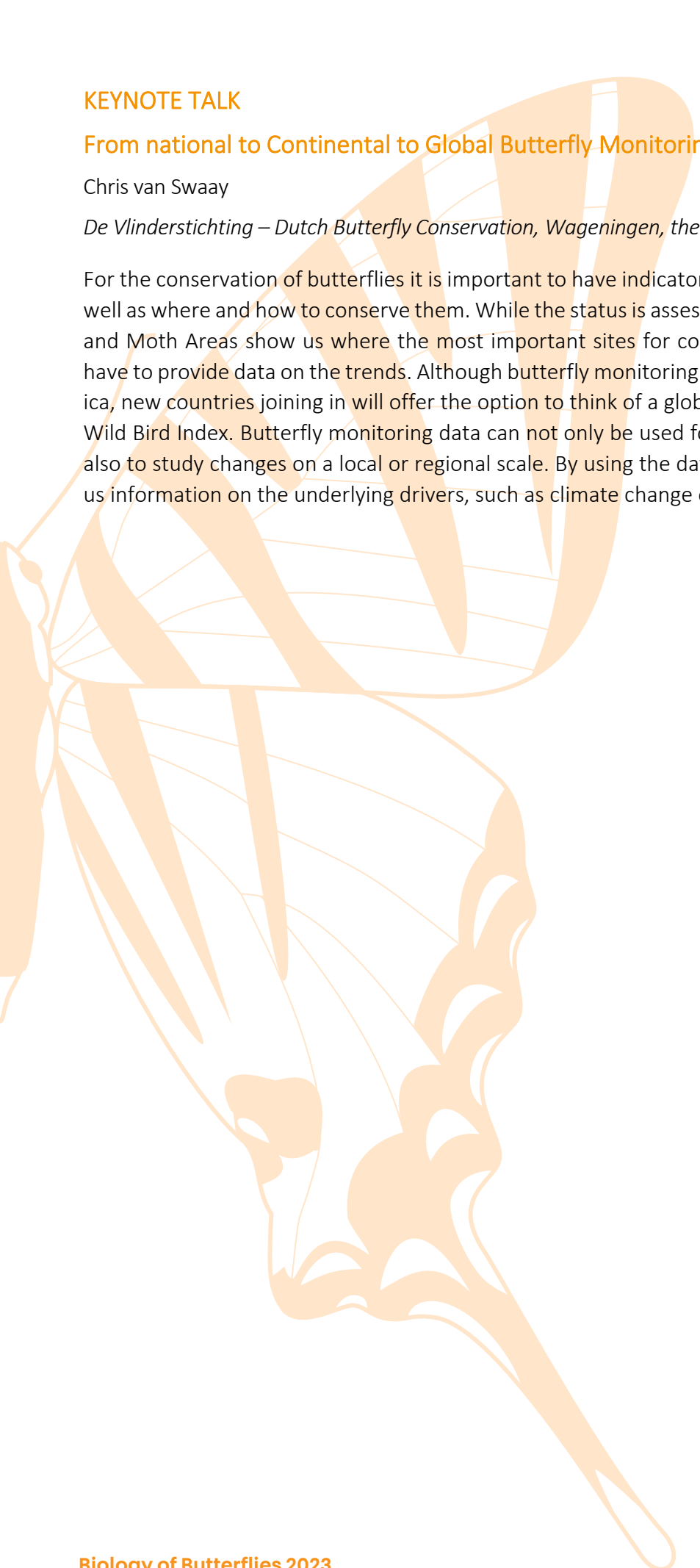
We discuss key challenges for BMS-IL, such as the difficulty to expand monitoring into the desert and in farmland areas; and the possible potential of the 15-minute protocol to address these challenges. Finally, we discuss exciting opportunities for international cooperation to advance science, nature conservation, and the public's involvement in both.

Long-term butterfly monitoring reveals climate-driven community restructuring with tropical species gains in a subtropical Hong Kong reserve

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Shifts in butterfly assemblage and abundance are widely observed and suggested to be a result of climate-driven species redistribution. While such shifts are evident in temperate regions, whether similar changes are occurring in tropical or subtropical butterfly communities is unclear due to the paucity of long-term monitoring data. Here we used 13 years of survey data at Fung Yuen Butterfly Reserve, Hong Kong to examine changes in a subtropical butterfly community. We calculated a community temperature index (CTI) based on species thermal affinities derived from a southeast Asian species occurrence database, which was compiled from the Global Biodiversity Information Facility and various other reputable databases and publications. From 2008 to 2020, CTI increased by 0.38°C with significant trends in spring and winter, concomitant with a rise in annual mean temperature by 1.07°C over the same period. Investigation of community dissimilarity revealed that species gain, but not species loss, was dominant and increasing over time. Major species gained reflected new species records in Hong Kong, which had warmer thermal affinities than species lost. Modelling species abundance to remove effects of covariation in species habitat preference on CTI, we showed that while habitat changes might have a role to play, climate warming was significant in restructuring species assemblage and abundance. Our results are consistent with predictions of how subtropical butterfly communities may shift under the warming climate. Long-term monitoring data in tropical ecosystems are important to detect community restructuring and understand warming impacts in this biodiverse region.

A large, stylized illustration of a butterfly in shades of orange and white, positioned diagonally across the page. The butterfly's wings are spread, showing intricate patterns of lines and shapes. It serves as a background element for the text.

KEYNOTE TALK

From national to Continental to Global Butterfly Monitoring

Chris van Swaay

De Vlinderstichting – Dutch Butterfly Conservation, Wageningen, the Netherlands

For the conservation of butterflies it is important to have indicators which show us the status and trend, as well as where and how to conserve them. While the status is assessed via Red Lists, and Important Butterfly and Moth Areas show us where the most important sites for conservation are, butterfly monitoring will have to provide data on the trends. Although butterfly monitoring is especially done in Europe and N America, new countries joining in will offer the option to think of a global butterfly indicator, comparable to the Wild Bird Index. Butterfly monitoring data can not only be used for national or continental indicators, but also to study changes on a local or regional scale. By using the data in community indicators they can give us information on the underlying drivers, such as climate change or land-use.

Citizen science approaches for developing Butterfly Monitoring Schemes in Europe

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The current biodiversity and climate crisis's are seriously impacting insects, but there are still gaps in our knowledge about their trends and threats. Long-term monitoring datasets are essential to clarify the trends of biodiversity and support evidence-based conservation actions. The European Butterfly Monitoring Scheme (eBMS) coordinates the long-term and standardized monitoring of butterflies across Europe, with 32 Butterfly Monitoring Schemes across 28 countries. This unique network of monitoring butterflies is consolidated based on the power of citizen science. Thousands of volunteers record butterflies every year helping science and conservation to provide robust data and trends of the butterfly populations. In this talk, we will present how the citizen science network of eBMS has been built and the basis to support a long-term monitoring community. Moreover, we will discuss the importance of a great coordination team, at European, national and regional level to provide a powerful and cost-effective network. Finally, we will share the lessons learnt during the improvement of the network, focusing on how to deal with different cultures, economies and butterfly diversities. With this talk, we intend to provide ideas and solutions made by eBMS to increase the interest on butterfly monitoring that could be applied globally.

VielFalterGarten – Citizen Science for monitoring and conserving urban butterfly populations

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There is growing public awareness and concern about insect declines, indicating both on the need of action but also on public's interest in doing so. Butterflies, and citizen science, offer two opportunities to do so. The Citizen Science project "VielFalterGarten" (word game: many-butterfly-garden or diversity-garden) is an education-and-action project that seeks to bring butterflies back to the city of Leipzig, jointly with the city council and BUND (Friends of the Earth Germany). Citizens can engage by monitoring butterflies, as well as by taking conservation measures in their gardens. Through educational materials and events, citizens learn about the ecology of butterflies. By actively observing different species by the 15-minute protocol, citizens not only enhance their knowledge of species and their needs, but also strengthen their nature relatedness, their self-efficacy, and overall pro-environmental behavior. Nature restoration activities with the VielFalterGarten community help butterflies but also result in greater knowledge of insect-friendly gardening measures and better acceptance of the city's implementation of restoration measures in parks. The 15-minute protocol serves as an easy-to-learn beginner's method that allows any interested citizen to participate in biodiversity monitoring. The monitoring data gathered by the citizen scientists serves to better understand the impacts of green-space management on butterflies, in private gardens, communal gardens and parks. We discuss the joint benefits for people and nature, and the huge potentials for expansion and networking among similar projects and initiatives.

Integrating DNA barcodes into Butterfly Monitoring Network

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DNA barcoding is a cost-effective molecular technique in identifying taxa and discovering cryptic species since its establishment two decades ago. The butterfly is the group of insects that benefit from this technique most, and many taxonomic problems have been solved, leading to better conservation status in the near future. The novel phylogenetic analytic tool even allows us to use the DNA barcoding data with genomic data to obtain high-resolution dated trees for conservation under the improved Evolutionarily Distinct and Globally Endangered (EDGE2) protocol. Compared to American and European countries, China's DNA barcoding data are still limited, despite its mega-diversity of butterflies in Asia (2500+ species). Data deficiency has become an obstacle in formulating effective conservation strategies. Using some recent achieved research cases involving DNA barcoding and taxonomic changes, we propose integrating DNA barcoding into the butterfly monitoring network, and make the data openly available to the science community worldwide. The undergoing Lepidoptera DNA barcode database project of Yunnan University (Kunming, China) derives from the routine molecular taxonomy work on butterflies in Yunnan Province (a global biodiversity centre in Asia), which would ultimately cover over 50% (1300+) of the country's total species count. Our philosophy is to establish a free open-access database with the data repository of NCBI GenBank with more detailed taxon information that would be useful to ecologist and conservationists. The database also welcomes data from other parts of China, and adjacent regions in South and Southeast Asia.

Social media records hold valuable information for conservation planning

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Citizen science plays a crucial role in helping monitor biodiversity and inform conservation. With the widespread use of smartphones, many people share biodiversity information on social media, but this information is still not widely used in conservation. Here, focusing on Bangladesh – a tropical mega-diverse and mega-populated country, we examine the potential importance of social media records in conservation decision-making. We show that adding Facebook data to the Global Biodiversity Information Facility (GBIF) data improved the accuracy of conservation planning assessments by identifying additional important conservation areas in the northwest, southeast and centre parts of Bangladesh, extending priority conservation areas by 2000–5000 km². Community efforts are needed to drive the implementation of the ambitious Post-2020 protected area targets, especially in mega-diverse tropical countries with a lack of reliable and up-to-date species distribution data. We highlight that conservation planning can be enhanced by including available data gathered from social media platforms.

Butterflies of western North America: trends, stressors, and prioritization for conservation

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Western North America includes centers of great biodiversity, hotspots of climate change, and intense agricultural and urban development. We will present a synthesis of ongoing research in the region to assess butterfly biodiversity change, uncover factors driving population declines, and prioritize species for conservation. With long-term monitoring and participatory science data, we find that many widespread species are becoming less abundant, while many narrow-ranged endemics are lacking in data needed to assess trends. As causal drivers of decline, the footprints of climate change, habitat loss, and habitat degradation are all apparent, but much yet remains to be learned about how these factors interact and how they might be mitigated. We also caution that the estimated rates of decline in butterfly abundance in western North America are derived from monitoring programs, which typically do not capture sufficient landscape-level variation to fully understand the effects of anthropogenic stressors on biodiversity change. Among the many challenges to estimating trends and disentangling drivers are heterogeneous data sources and ubiquitous declines that create uncertainty when modeling species-specific traits associated with demographic trends. By combining multiple lines of evidence linking species traits, stressors, and biodiversity change, we present a relative ranking of species at risk to motivate conservation as well as political action.

Spatial, temporal, and taxonomic trends from butterfly monitoring programs

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In 1976, Ernest Pollard initiated what has become the longest, ongoing effort to monitor population trends in butterflies. Since then, many butterfly monitoring programs have been established globally. This expansion positions butterflies as a key taxonomic group to better understand insect demographic trends amid a potential “insect apocalypse”. Assessing different monitoring programs is therefore key to coordinate efforts towards a global butterfly monitoring network, which will in turn be fundamental to inform butterfly conservation moving forward. I will present analyses that synthesize thousands of population time series collected globally, focusing on (i) the spatial distribution of the populations currently assessed; (ii) the temporal coverage of different populations; and (iii) how these spatio-temporal trends vary depending on different taxonomic groups under consideration. These analyses identify clear gaps in our understanding of butterfly population trends, but most importantly, provide useful directions to optimize the expansion of ongoing butterfly monitoring initiatives.

We acknowledge the support of contributing Butterfly Monitoring Schemes and of the IUCN Species Survival Commission butterfly and moth specialist group.

On the detectability of climate change impacts on insect populations: An individual-based simulation approach to explore monitoring design and variation in weather effects

Katherine L. Bell, Matthew L. Forister

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Long-term monitoring of butterfly populations is frequently used to understand how insects are responding to climate change. A relatively unexplored issue is the extent to which weather effects are reliable indicators of biological effects caused by variation in abiotic conditions. We used an individual based simulation model to evaluate three monitoring designs and asked how effectively impacts of weather on survival can be recovered. We modeled a univoltine butterfly population, with individuals distributed on a landscape of patches mimicking the distribution of our study system in the northwest USA. We used daily maximum temperatures from corresponding locations of patches from the PRISM climate database from 1981 – 2021 to impose an impact on larval survival that varied among years and patches. Weather for the days that an individual was in their larval stage was summarized and probability of survival calculated using a logit link function. We varied values for beta (the impact on survival) and the intercept (from a binomial model) to impose variable impacts of weather. Next, we “surveyed” adults using three different methods: a single count during peak flight of adults, a biweekly count of adults throughout the flight season, and biweekly presence/absence observations throughout the flight season. We ran linear models for these data and asked how well we recovered the “true” impact of maximum temperatures on butterfly populations. Results will be discussed in the light of monitoring design and strength of inference that should be expected from empirical studies with a similar data structure.

Conservation of butterflies and their habitats

KEYNOTE TALK

Mark-release-recapture of eleven butterfly species unravels different conservation needs

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For successful conservation, the species you want to protect must really be understood. We therefore studied a total of eleven butterfly species in large-scale mark-release-recapture studies from 2015 to 2021 in a nature reserve in eastern Brandenburg (Germany). Each of the species was studied in one to five years. Every captured individual was marked with a unique code on its wings and its sex was recorded. During each capture and recapture event, the animal's position, wing condition, behaviour and (if applicable) nectar source use were recorded. The data allowed us to assess for each species: the numbers of individuals and their phenologies, their distributions in space, movement patterns and dispersal abilities, their behaviours and flower uses. Thus, the differences among species as well as within species among generations and years could be determined. Overall, an increase in the number of individuals was observed over the study period. The expression of proterandry was very different among species and sometimes differed greatly between generations but not years. Dispersal ability was highly variable among species, but also whether males or females were the more expansive sex, which has a great influence on the connectivity potential between populations. Behaviour and nectar plant use were very different among species, generations and sexes. This implies very different habitat requirements of the species with their sexes and generations, all of which must be met to ensure the long-term survival of all.

How much “nature” do we need in agricultural landscapes to preserve butterflies?

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Large-scale monitoring programmes help us understand the patterns and mechanisms of ongoing insect decline. We analysed butterfly data from the first four years of a governmentally funded insect monitoring system that was started in 2018 in Baden-Württemberg, Germany. Altogether 200 sites, including 40 nature reserves, 80 arable sites and 80 grassland sites were randomly selected in a spatially balanced manner. Sites are surveyed in a 4-year rotational cycle, each year 50 sites are sampled. Butterflies and burnet moths were counted four times between mid-May and end of July along 1.5 km long transects using Pollard-walks between 2018 and 2021. Landscape composition was assessed within a 100m buffer around each transect. Relationships between landscape variables and abundance, species richness and diversity of butterflies (all species and different subsets of red list species) were analysed using GLMMs.

We found the proportion of protected semi-natural open biotopes (e.g. calcareous grasslands, wet meadows, hedgerows etc.) as the most influential landscape variable having a positive effect on butterflies. A simple breakpoint analysis showed that butterfly abundance almost doubled between 0% and 10% cover of protected semi-natural open biotopes, while species richness showed a similar increase between 0% and 15%. Further landscape variables such as proportion of forests, landscape diversity, altitude and proportion of high nature value farmland were also positively related to some butterfly variables, but only in some species subsets. Our analysis mostly covered normal landscapes and rather common species, for rare species and nature reserves specific habitat management is mandatory.

Restoring habitats of the myrmecophilous butterfly *Phengaris (Maculinea) teleius* on former agricultural fields by sod translocation

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Many restoration projects are performed to reverse the immense loss of natural grassland area in Europe by creating suitable abiotic conditions. However, more is needed when restoring wet meadows for the butterfly *Maculinea teleius*. This species engages in a complex myrmecophilous relationship with one host plant, *Sanguisorba officinalis*, and one obligate host ant, *Myrmica scabrinodis*. First, starting conditions on former agricultural land were optimized including modification of the local hydrology, removal of the fertilized topsoil, application of liming, and translocation of fresh clippings as a seed source. The second part involved a sod translocation experiment to create islands of habitat to promote host plant and host ant colonization. After 4 years following the restoration, plants spread from the transplanted sods to the surroundings. The vegetation composition and structure of the transplanted sods attracted colonization of *Myrmica* ants into the restored areas. Following the increase in vegetation cover and height, *Myrmica* ant colonies further spread into the restored areas. Therefore, sod translocations can be considered an effective restoration method following topsoil removal in the process of restoring wet meadows to provide a starting point for ant colonization and plant dispersion. The results of the experiment contribute to the evidence-based restoration of wet meadows, including complex interactions between invertebrates and their ecological relationships.

The value of novel habitats for conservation of butterflies – a short review

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With decreasing area and number of natural habitats the conservation of butterflies may be supported by presence of novel habitats. Novel habitats are significantly impacted by human activities, leading to the emergence of new ecological processes and thus new species compositions.

For example, several studies in novel habitats such as road verges, railway embankments, quarries, gravel-pits, ruderal post-industrial areas have proven that these habitats may have high butterfly diversity. Explanation for this may be that these habitats have specific plant composition including rare, early successional plant species that may be both nectar source and host for larvae. These plants may bloom at different times of the year extending the period during which butterflies can feed and reproduce. Additionally, novel habitats may contain microhabitats that are not present in natural habitats, such as rocks, areas of bare ground or other structures that provide shelter for butterflies during inclement weather.

However, it is important to note that not all novel habitats are beneficial for butterflies, and some may even pose a threat to their survival. For example, novel habitats that contain invasive species, pollutants or salt influx that may impact larvae development. Also, road-mortality may negatively impact suitability of road verges. Only few studies examined population stability, reproduction and long-term survival of butterfly populations in novel habitats compared to more natural ones. Therefore, it is essential to carefully study and manage novel habitats to ensure that they provide value for conservation of butterflies.

Conserving Madeira's threatened endemic butterflies

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Madeira is a hotspot of threatened endemic butterflies in Europe. According to the most recent European Red List of Butterflies, two species, the Madeiran Speckled Wood (*Pararge xiphia*) and the Madeiran Brimstone (*Gonepteryx maderensis*), are listed as endangered, and one, the Madeiran Large White (*Pieris wollastoni*) even as Critically Endangered (Possibly Extinct).

In the course of a LIFE4BEST-funded project, intensive surveys along 534 km of survey routes across the whole island were carried out in 2021 and 2022 with the help of volunteers.

Pararge xiphia turned out to remain one of the most common species, despite its decline after the introduction of the Speckled Wood (*Pararge aegeria*) in the 1960s. However, its main strongholds are confined to undisturbed laurel forests in the centre of the island, whereas the invasive congener predominates in coastal areas. In contrast, *Gonepteryx maderensis* has become a low density species, and only a few major breeding sites remain near steep cliffs within the laurel forest, which harbour its larval foodplant, *Rhamnus glandulosa*. No sign was seen of *Pieris wollastoni*, which is now presumed extinct and thus becomes the first globally extinct European butterfly.

The talk discusses the reasons for the declines and possible steps to improve the conservation status of Madeira's endemic butterflies. Further monitoring is facilitated by newly set up transect routes, a recording app and training resources in multiple languages, which enable the involvement of citizen scientists, both from the local population and tourist visitors to the island.

How to promote the Southern White Admiral? – Scientific evidence for a species conservation plan

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In Central Europe, many butterfly species of open forests have been rapidly declining during the last decades. One of them is the Southern White Admiral (*Limenitis reducta*), which is nowadays among the rarest and most threatened butterfly species in Germany. Information on the species' ecology is scarce, though crucial for an evidence-based conservation. In a three-year study, we recorded habitat requirements, demographic parameters, population sizes and dispersal distances of *L. reducta* in two remnant populations. We used capture-mark-recapture methods as well as video tracking of larvae and repeated field observations. Furthermore, we implemented management measures such as small clear-cuts and the optimization of ride edges to bolster the populations.

Our results underpin the high conservation concern of *L. reducta* in Germany. Total population size of the largest population studied was estimated at 123 adults in the best of three years. Individual home ranges were typically small, only 9% of the individuals dispersed more than 1 km. Females preferred to oviposit on those *Lonicera xylosteum* bushes located in very sunny positions and characterized by a reduced vitality. Survival rates from the egg to the adult were positively affected by high solar irradiation but averaged only 3%. The number of overwintering larvae was positively affected by the habitat management measures.

We conclude that *L. reducta* requires a large network of suitable habitats. Such a network of sun-exposed habitat patches within forests can be created at low cost if foresters and conservationists collaborate and can promote a variety of open forest species.

Status and Conservation of a Rare, Endangered Great Basin Skipper (Nevada and California, USA)

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Western North America is experiencing rapid warming and drying under anthropogenic climate change. The region is also home to a number of declining butterflies, including the Carson wandering skipper (*Pseudocopaeodes eunus obscurus*), a narrow-ranged, endangered subspecies of skipper native to northwestern Nevada and northeastern California. Despite over 20 years of protection by the US Fish and Wildlife Service (USFWS) under the US Endangered Species Act, the Carson wandering skipper (CWS) continues to decline. In 2023, only 19 individuals were observed over the course of the flight season, compared to a recent high year of 2011 when 427 individuals were observed. Considering much that remains to be discovered about the ecological requirements of the taxon, making meaningful and actionable conservation decisions is difficult. While the other subspecies of *Pseudocopaeodes eunus* could be used to better understand the biology of the CWS, regular monitoring of those populations has not occurred and preliminary surveys over the last three years have failed to find any of the unlisted subspecies. Moreover, citizen science data is sparse, further compounding the difficulty of conserving the CWS. Here, we assess status of the CWS using historical and USFWS monitoring data, and characterize what habitat remains without assuming the remaining populations reside in optimal habitat. Finally, we consider how conservation and management can move forward with this and other species in the region where information is sparse and threats are compounding.

Genome structure and evolution

KEYNOTE TALK

Winging it: Understanding genome instability in *Polyommatus*

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Lepidoptera, the order of butterflies and moths, have a largely conserved karyotype of 31 chromosomes. Against this background of karyotypic stasis, a subset of species displays dramatic variation in chromosome organisation. Recently, high-quality chromosomal genome assemblies have been generated for over 200 species of Lepidoptera, including 188 by the Darwin Tree of Life project. We have used these to interrogate the processes that shape genome organisation in Lepidoptera. Phylogenetically-aware analysis of conserved single-copy orthologs mapped to chromosomes from 210 Lepidoptera and 4 Trichoptera (caddisflies; outgroup) was used to predict ancestral linkage group number and content across Lepidoptera. We find lepidopteran karyotypes have remained stable with most species having experienced no or just one fusion. We find the last common ancestor of Lepidoptera likely had 32 chromosomes, and that the apparent base number of 31 derives from an ancient fusion that occurred in the last common ancestor of the speciose clade Ditrysia. Against this stable background, we identify nine independent lineages that have evaded these constraints and undergone extensive reorganisation. Two instances of extreme genome reorganisation occurred in the blue butterflies, *Polyommatus*. This group includes *P. atlantica*, whose 228 chromosomes result from rampant fission events. Fission is not universal, as the Z chromosome remarkably withstands fragmentation. Together, these analyses demonstrate how chromosomally-contiguous genomes across Lepidoptera offer an unprecedented opportunity to explore the role of selective constraint in the evolution of genome structure.

What can we learn about chromosomal speciation in butterflies from studying Leptidea?

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It is not uncommon for butterfly species to differ in chromosome number. Some genera such as *Erebia*, *Polyommatus* and *Leptidea* take this to an extreme, with the wood white, *Leptidea sinapis*, showing the greatest intraspecific karyotype difference of any animal caused by numerous chromosome fusions and fissions. Despite this karyotype difference, F1 hybrids of extreme chromosomal races within *Leptidea sinapis* are fertile. This conflicts with traditional models of chromosomal speciation positing meiotic problems during F1 meiosis. However, it might be too early to rule out an association between karyotype evolution and speciation in *Leptidea*. F2 hybrids show reduced survival, likely due to recessive genetic incompatibilities. We mapped the genetic basis of hybrid inviability by contrasting allele frequencies in F2 hybrids that survived until imago with those that succumbed during development. Modern chromosomal speciation theory emphasizes recombination suppression in heterokaryotypes as a potential hotspot for species divergence. We know less about the effects of chromosome fissions and fusions, which are of particular interest in butterfly genetics. We observe that candidate regions involved in hybrid inviability are enriched close to the chromosome fusion breakpoints. We suggest that rapid fixation of rearrangements combined with low recombination rates may have contributed to making breakpoint regions hotspots of genic incompatibilities. These results provide experimental evidence that karyotype evolution in butterflies is associated with the evolution of reproductive isolation, which has widespread implications when interpreting patterns of karyotype evolution in butterflies.

Recombination rate differences as a consequence of chromosomal rearrangements

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Reshuffling of genetic material allows novel allele combinations and decouples genetic variants with opposite selective coefficients and linked neutral variants. This reshuffling occurs by independent assortment of chromosomes and by homologous recombination. The amount of reshuffling is affected by the size and number of chromosomes, but studies of these effects in natural populations are rare. Repeated chromosome fusions and fissions in the wood white butterfly (*Leptidea sinapis*) have resulted in almost twice as many chromosomes in the southern population compared to the northern populations. We used linkage maps to characterise the global and regional recombination rate variation in two populations with distinct karyotypes and to assess the relationship between chromosomal rearrangements, crossing over and maintenance of genetic diversity. We found an effect of karyotype on both global and regional patterns of recombination rate variation. As expected, the population with a larger chromosome number had a higher recombination rate. However, the difference was smaller than expected due to a greater number of double crossovers in the longer chromosomes. Both populations showed an association between regional recombination rate variation and genetic diversity and we also found an association between the intergenic diversity and recombination rate differences between the two populations. In conclusion, chromosomal rearrangements affect global and regional recombination rates, subsequently influencing the maintenance of genetic diversity.

Genome architecture in the holocentric silkworm *Bombyx mori* and related species

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Genome organization in eukaryotes follows conserved principles such as the formation of Chromosome Territories (CTs), chromatin compartments, and topologically associated domains (TADs). Yet, most of these concepts derive from analyses of organisms with monocentric chromosomes, while centromeres also strongly impact the spatial genome organization. Thus, to challenge the universality of folding principles, we investigate the 3D genome architecture of an organism with holocentric chromosomes, the silkworm *Bombyx mori*. We found that, at the genome-wide scale, *B. mori* chromosomes are highly territorial and lack trans contact patterns. Additionally, as described in other eukaryotes, *B. mori* chromosomes segregate into an active A and an inactive B compartment. Remarkably, we also identify a third compartment with a unique contact pattern encompassing ~20% of the genome that we named X. Compartment X shows strong enrichment of short-range interactions and depletion of long-range interactions. It hosts a specific combination of genetic and epigenetic features and localizes at the periphery of CTs. Whether these observations are specific to *B. mori*, more largely spread among Lepidoptera species or even related to the holocentric way of life is still unclear. To answer these questions, we started the investigation of the 3D architecture of a broader range of species using publicly available Hi-C datasets. We already found that the peculiar X compartment described in the silkworm can be found in other moths and butterflies. Thanks to the high synteny among Lepidoptera chromosomes, we are now investigating the possible conservation of the genomic regions giving rise to such 3D pattern.

Strange genome architecture in ghost moths (Hepialidae)

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Vast majority of moths and butterflies (Lepidoptera) typically have $2n=62$ holocentric chromosomes with a female heterogametic WZ/ZZ system. The W chromosome should have evolved later in the their evolution as it is present only in Tischerioidea and non-tineoid Ditrysia.

We performed combined cytogenetic and genomic analyses in five representatives of the early diverging superfamily Hepialoidea, namely *Endoclita excrescens*, *Hepialus humuli*, *Korscheltellus lupulina*, *Phymatopus californicus*, and *Triodia sylvina*. Hepialids have extremely large genomes, size of which ranged between 1.58–3.08 Gbp. Yet, their repeat content was comparable to that observed in other Lepidoptera (45–60%). Cytogenetic analyses revealed chromosome number $2n=64$ in both sexes of all species, but some females of *P. californicus* with $2n=63$ suggesting their polymorphic sex chromosome constitution. Remarkably, mitotic chromosomes had clear primary constriction, although early anaphase nuclei showed parallel separation of chromatids typical for holocentric chromosomes. The absence of differences in genome size and chromosome number between the sexes suggests the presence of W chromosomes in all species, which was confirmed by DAPI staining of pachytene nuclei. However, genomic *in situ* hybridization failed to detect the W chromosomes in *T. sylvina* and *H. humuli*, which indicates similarity between repeat landscapes of their Ws and autosomes. Moreover, the hepialid W chromosomes did not form a female-specific heterochromatin used as a proxy of W chromosome across Lepidoptera. The Z-linked gene content was conserved between hepialids and ditrysiian Lepidoptera.

Our results shed new light on our understanding of sex chromosome evolution in Lepidoptera and questions holocentricity of their chromosomes.

Insights into early stages of sex chromosome evolution from a novel neoW chromosome in the African Monarch Butterfly, *Danaus chrysippus*

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Heteromorphic sex chromosomes are often highly degenerated, but the progression of this degeneration is not well understood. Recently, it has been discovered that a female sub-population of *Danaus chrysippus* (African Monarch) has undergone a chromosome fusion event, causing an autosome to become a non-recombining neoW sex chromosome, which additionally cosegregates with the male-killing endosymbiont, *Spiroplasma*. This novel sex chromosome is very young compared to other studied examples and provides an excellent opportunity to study the early stages of sex chromosome evolution. We compared highly contiguous assemblies of the neoW and its autosomal homolog and tested for signs of degeneration and differentiation by searching for premature termination codons in the genes present on the two chromosomes. We then used population data to identify individuals carrying the neoW and/or *Spiroplasma*, and performed genomic divergence analysis using a kmer-based approach. Differentiation between the neoW and its autosomal counterpart, and accumulation of repetitive elements, would indicate that the neoW chromosome is already on a trajectory to becoming a fully differentiated sex chromosome, though whether any such signal can be seen in an extremely young system is an unanswered question. The neoW sex chromosome contains genes involved in regulating wing pattern colouration, which further provides unique insight into the genetic basis of this complex trait and how it can be influenced by other structural factors. Our study fills an important gap in the understanding of sex chromosome evolution and degeneration of non-recombining genomic regions.

Neo-W degradation locally rescued by rare recombination events in the Spanish marbled white butterfly

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Neo-sex chromosomes represent a powerful opportunity to study the mechanisms underlying the evolution of Y and W chromosomes. Iberian populations of the butterfly *Melanargia ines* have recently evolved a pair of neo-sex-chromosomes via a Z-autosome fusion. As female Lepidoptera undergo achiasmatic meiosis, the unfused autosome that co-segregates with the W chromosome is female-limited and non-recombining — it is effectively a neo-W chromosome. Lacking recombination, the neo-W is expected to degenerate rapidly through Muller's ratchet and reduced efficacy of selection (i.e. hitchhiking of deleterious and beneficial mutations). We infer that the *M. ines* neo-sex chromosomes arose in Iberia after or concurrently with the split between Iberian and North-African populations around 1.7 million years ago. We find that the neo-W shows signs of degeneration: it has accumulated premature stop codons and indels in exons, and has a reduced efficacy of purifying selection (π_0/π_4) compared to the neo-Z and autosomes. Surprisingly, we identify a 2 Mb region at one end of the neo-W that is both less diverged (from the neo-Z) and less degraded than the rest of the chromosome, suggesting a history of rare genetic exchange between the two neo-sex chromosomes. We discuss potential mechanisms of genetic exchange between the neo-W and the neo-Z, which include rare recombination events in females or a temporary neo-W escape in males. Recombined neo-W haplotypes subsequently swept in females, as neo-Z-derived regions are not degraded. Overall, our results suggest that neo-W degradation can be locally rescued by rare genetic exchange between neo-W and neo-Z.

Assembly and characterization of the W chromosome in monarch butterfly (*Danaus plexippus*)

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We report a novel genome assembly for the monarch butterfly (*Danaus plexippus*), focusing on the W chromosome. This species harbors a neo-Z chromosome, arising from the fusion of the ancestral Z with an autosome. Previous cytogenetic analyses indicated a similarly large and bipartite W chromosome, suggesting the possibility of a comparable neo-W, but much ambiguity remains concerning the sequence and history of monarch W. We generated PacBio HiFi reads with Hi-C data from females to support *de novo* assemblies of maternal and paternal haplotypes using Trio binning. Putative W contigs from the maternal genome were determined based on male to female coverage and sex specific kmers. The paternal assembly and W contigs were scaffolded using Hi-C data. The W chromosome scaffold length was around 10Mbp, thus leaving about one third of this chromosome in unscaffolded contigs. While primarily composed of transposable elements (TEs), the W contains at least three protein coding genes that arose either through retroposition or ectopic recombination from other chromosomes. The prevalent repetitive content of the W chromosome are elements from the LINE and LTR retrotransposon groups. Surprisingly, the TEs on W chromosome have lower divergence compared to rest of the genome, suggesting their ongoing accumulation. Population genetic analysis indicates dramatically reduced levels of heterozygosity on the W chromosome. Finally, despite this novel W assembly, strong evidence for or against a neo-W chromosome origin remains elusive.

A novel sex determination mechanism in a satyrid butterfly

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The mechanisms of sex determination in insects are extremely variable, and therefore model organisms do not necessarily represent the taxonomic group to which they belong. In Lepidoptera, sex determination is best described in the silkworm, where the presence of a W-chromosome-linked piRNA called *Feminizer* leads to cleavage of the Z-chromosome-linked *Masculinizer* (*Masc*) gene mediated by a ping-pong mechanism. The fate of *Masc* (cleaved or intact) then sets female or male development in motion through the downstream genes of the sex determination cascade. The question remains how widespread the silkworm sex determination mechanism is in Lepidoptera, since basal clades often lack W chromosomes and secondary loss of the W chromosome has also been found in several species. The recent discovery of W loss in the Afrotropical butterfly *Bicyclus anynana* encouraged us to investigate the characteristics of *Masc* in this species, which led to the discovery of a novel sex determination mechanism in this butterfly. *Masc* contains a hypervariable region in *B. anynana* which is exclusively heterozygous in adult males because all homozygous males die at the end of embryonic development. This lethality is caused by lack of dosage compensation and the inability to produce the male *doublesex* splice variant. The system shows striking similarities to complementary sex determination (*csd*) in honey bees, where homozygosity at the *csd* locus results in sterile males who are killed by workers. Furthermore, both *B. anynana* and the honey bee possess extreme allelic variation at these sex-determining loci and are under very strong frequency-dependent balancing selection.

Vision and signalling in butterflies

Sexually dimorphic pierid eyes

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Flower-visiting butterflies have sophisticated color vision. Their eyes are spectrally rich with at least three, usually six or eight, or even more than ten distinct spectral receptors. These spectral receptors are housed in the ommatidia, each bearing nine receptors, in three fixed combinations, making the eyes a patchwork of three spectrally distinct ommatidia types. The ommatidia types are often anatomically distinguishable by the various pigments in the eye, which act as spectral filters.

We first noticed the eye's sexual dimorphism in *Pieris rapae* (Pierinae): we found ommatidia that fluoresce under violet illumination in males, but not in females. The fluorescent material absorbs the violet light, changing the violet receptors into double-peaked blue receptors in males. This finding stimulated us to explore the other pierid subfamilies. We thus found that the red receptor of *Colias erate* (Coliadinae) peaks at 640 nm, but the females in addition have 620 nm- and 660 nm-peaking red receptors. The diversity is attributed to the sexual dimorphism of the reddish screening pigments. *Lepitidea amurensis* (Dismorphiinae) is peculiar: their eyes are spectrally simple in both sexes, but the ventral eye part appears rough only in males because that part is a random collection of large and small facets.

More recently, different forms of sexual dimorphism have been found in Lycaenids and Nymphalids, indicating that the phenomenon is more common among butterflies than previously thought. What and how drives its evolution will be a central topic of butterfly vision research in the next decade.

Sex-linked gene traffic underlies the acquisition of sexually dimorphic UV color vision in *Heliconius* butterflies

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The acquisition of novel sexually dimorphic traits poses an evolutionary puzzle: how does a new trait arise and become sex-limited? Recently acquired color vision, sexually dimorphic in animals like primates and butterflies, presents a compelling model for understanding how such traits become sex-biased. For example, some *Heliconius* butterflies uniquely possess UV color vision, which correlates with the expression of two differentially tuned UV-sensitive rhodopsins, UVRh1 and UVRh2. To discover how such traits become sexually dimorphic, we studied *H. charithonia*, which exhibits female-specific UVRh1. We demonstrate that females, but not males, discriminate different UV wavelengths. Through whole-genome shotgun sequencing and assembly of the *H. charithonia* genome, we discovered that UVRh1 is present on the W chromosome, making it obligately female-specific. By knocking out UVRh1, we show that UVRh1 protein expression is absent in mutant female, and wildtype male, eye tissue. A PCR survey of UVRh1 sex-linkage across the genus shows that species with female-specific UVRh1 expression lack UVRh1 gDNA in males, confirming W-linkage as the mechanism behind dimorphic expression. Moreover, both this event, and mutations leading to differential UV opsin sensitivity, took place early in the history of *Heliconius*. Thus, this dichotomy of sexually dimorphic and monomorphic duplicate photoreceptors evolved due to sex linkage rather than via evolution of female-specific regulatory elements. Our results suggest a third path for the acquisition of sexual dimorphism, which is inconsistent with existing mechanistic models. We propose a model where sex-linked gene traffic genetically partitions the trait by sex before the shift in UV sensitivity.

KEYNOTE TALK

Design, function and evolutionary patterns of red receptors in nymphalid butterflies

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The eyes of many butterflies contain photoreceptors with peak sensitivity in the orange and red part of the light spectrum, which form the basis for the discrimination of red hues. Physiological and anatomical evidence for these »red« receptors has been so far missing for the largest butterfly family, the brush-footed butterflies (Nymphalidae). We have discovered that the retina of many nymphalids contains the red-sensitive photoreceptors, which are usually the basal cells R9 in the red-shining ommatidia. The eyes with red ommatidia are regionalized, either having a dorso-ventral gradient of ommatidial types in larger butterflies, or sharply delineated dorsal and ventral zones in smaller butterflies. Using intracellular recordings, optical measurements and anatomical analysis, we have shown that the red ommatidia have rhabdoms lined with granules with a red filtering pigment and contain unusual cells R1&2 that likely express a LW opsin. These green-sensitive R1&2 are postsynaptic to the red-sensitive cells and together form a colour opponent pair. In the tribe Melitaeini, however, the red receptors are allocated to the cells R3-8 and seem to have evolved independently. In the tribes Argynnini and most of Melitaeini, the red receptors are found only in males. The cells that constitute the red-green opponent pairs are probably ancestral to nymphalids, but seem to have been switched off in entire clades and in females of distinct taxa. The phylogenetic patterns of retinal organization are a clear demonstration of the remarkable evolutionary flexibility of butterfly compound eyes.

Ecology and phylogeny of butterflies as seen through the eyeshine

Primož Pirih

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The apposition eye shine of butterflies is analogous to the (usually reddish) eye glow observed in the dark-adapted superposition eyes of many moths, but it is less well known because its observation requires axial illumination provided by an opthalmoscope or an epi-illumination (=material) microscope. The eye shine is due to the light reflected at the tracheolar tapetum – a multilayer at the base of ommatidia. The reflections from individual ommatidia depend on the multilayer tuning, the length of the rhabdom, the presence of red screening pigments and the absorption spectra of the photoreceptor visual pigments – different species offer a whole rainbow of ommatidial reflections.

The eyeshine disappears due to the pupil closure in 2~15 seconds. The pupil speed is likely related to the visual environment of the species. The spectral sensitivity of individual pupils reflects the presence of UV and blue photoreceptors. The change of reflection spectra in the first second, due to visual pigment isomerisation (rhodopsin-metarhodopsin), can be recorded with a microspectrometer, yielding estimates for the main (green-peaking) visual pigment. In nymphalid butterflies, the absorption peak of the main visual pigment is correlated with the presence of the red (ommochrome) screening pigment: the main rhodopsin usually peaks at ~520 nm (*Vanessa*, *Apatura*, *Hipparchia*), but shifts to ~540 nm when the red screening pigment is present (*Morpho*, *Argynnis*, *Melitaea*).

Optical measurements from living butterfly eyes are relatively easy and can be performed using DIY equipment, offering a wider application potential within the frameworks of (visual) ecology and phylogeny.

Strong phylogenetic inertia drives diversification of eye size in butterflies

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Quantifying the mode, tempo and drivers of trait evolution is a central tenet in evolutionary biology. Sensory organs offer a unique opportunity to address these key questions as these traits, especially eyes, can play a crucial role in shaping animal lifestyles.

Mounting evidence suggests that diel activity is a major driver of eye design in animals, but other factors are seldom explored. Furthermore, diurnal versus nocturnal animals experience dramatically different light conditions and can mask differences in eye adaptations within diurnal lineages, for example, those occupying different habitats. To address this gap, for the first time, we quantify mode and tempo and drivers of eye size evolution in butterflies in a macroevolutionary framework.

We generated 3D micro-CT scans of 460 individuals across 59 species of European butterflies for measuring eye surface area. Firstly, after accounting for phylogeny, there was strong allometric scaling between eye surface area and body size (forewing length) and males generally had larger eyes than females. Phylogenetic comparative analyses further suggested that size-corrected eye surface area had a strong phylogenetic signal and followed a Brownian motion process. Furthermore, homogenous-rate Brownian motion models had a better fit than variable-rate models and predictors (habitat, male mating strategy, sexual dichromatism, hostplant quality) did not affect eye size evolution.

Overall, phylogenetic inertia, not ecology and behaviour, is a major driver of eye size evolution in temperate butterflies which is in stark contrast to the widely held belief. The consequences this has on community assembly remain an exciting arena.

Divergence of visual acuity in *Heliconius* butterflies

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Divergence in visual systems has been extensively studied in neotropical *Heliconius* butterflies, which rely on visual signals for foraging and finding mates. However, acuity, the ability to resolve spatial features has not previously been measured in these butterflies. We assayed visual acuity and visual performance in *Heliconius erato*, including *H. e. lativitta* and *H. e. cyrbia*, found in rainforests to the east and west of the Ecuadorian Andes, respectively. To further explore the links between ecology and divergence across lineages, we tested the parapatric species, *H. himera*, which is found in dryer, more open Andean forests in southern Ecuador.

Visual acuity was estimated from both, eye anatomy measures and by scoring the behavioral response to stripes in motion, i.e., optomotor response. The acuity values described in this study (~ 0.50 cpd) may help distinguish visual cues that are relevant to conspecific signaling and navigation in *Heliconius*. We found heritable differences between species, with higher visual acuity in the *H. erato* populations, which inhabit more visually complex forests. Additionally, we found higher acuity in male *Heliconius*, indicating differential perception between the sexes. This provides compelling evidence for sensory adaptation in *Heliconius* and highlights the impact of disparate environments and lifestyles on vision.

Potential evolutionary tradeoffs between the development of visual and central nervous systems among butterflies

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The development and maintenance of nervous tissue is metabolically expensive. There is evidence that some species of butterflies perform visually guided food or mate choice or preference learning. We investigated potential evolutionary tradeoffs between peripheral and sensory nervous systems among butterflies, and their relationship to concomitant evolution of wing pattern complexity. Using published raw measurements, we calculated the relative sizes of the peripheral and nervous system of 40 species of butterflies. Species included representatives of 4 families: the Nymphalidae, Lycaenidae, Pieridae, and Papilionidae. Additionally, we used the Nymphalid wing *bauplan* to score the complexity of the ventral and dorsal wing patterns of males and females of each of these species. We performed ancestral state reconstructions of the relative sizes of each of these brain regions. We also performed a reconstruction of the evolution of wing complexity. We found that, in *Heliconius*, the central brain and mushroom body calyx has evolved to be larger, while the optic lobe and antennal lobes have evolved to be relatively smaller compared to other genera in Nymphalidae and compared to other families. Additionally, we found that the antennal lobes have evolved to be relatively larger among Lycaenids. Furthermore, Lycaenids retain a relatively simple ancestral wing pattern, while Nymphalids possess the most complex wing patterns. Overall, we have revealed evidence of an evolutionary tradeoff between the visual and central nervous system across butterflies.

KEYNOTE TALK

The genetic basis of structural colour variation in mimetic *Heliconius* butterflies

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Structural colours, produced by the reflection of light from ultrastructures, have evolved multiple times in butterflies. Unlike pigmentary colours and patterns, little is known about the genetic basis of these colours. Reflective structures on wing-scale ridges are responsible for iridescent structural colour in many butterflies, including the Müllerian mimics *Heliconius erato* and *Heliconius melpomene*. Here we quantify aspects of scale ultrastructure variation and colour in crosses between iridescent and non-iridescent subspecies of both of these species and perform quantitative trait locus (QTL) mapping. We show that iridescent structural colour has a complex genetic basis in both species, with offspring from crosses having wide variation in blue colour (both hue and brightness) and scale structure measurements. We detect two different genomic regions in each species that explain modest amounts of this variation, with a sex-linked QTL in *H. erato* but not *H. melpomene*. We also find differences between species in the relationships between structure and colour, overall suggesting that these species have followed different evolutionary trajectories in their evolution of structural colour. We then identify genes within the QTL intervals that are differentially expressed between subspecies and/or wing regions, revealing likely candidates for genes controlling structural colour formation.

Adaptive exchange of a *regucalcin* drives the evolution of visual mating preference in *Heliconius* butterflies

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Variation in visual preferences is important for sexual selection and speciation, but little is known the underlying genetics. This is significant because identifying the genes involved is critical for our understanding of how behavioural differences are generated, both across evolutionary time and during development. In particular, hybridization has been suggested to play a role during behavioural evolution, but a direct link between specific loci and behaviours has not been made. *Heliconius* butterflies display bright warning patterns, which are also used as mating cues. We show that male preferences for red patterns segregate with a genomic region of increased admixture between red-preferring *Heliconius* butterfly species, indicating that adaptive introgression has contributed to the parallel evolution of visually-guided behaviours in these butterflies. Differences in neural expression of *regucalcin*, which coincides with this introgressed region, is associated with visual preference variation, and disruption of *regucalcin* with *CRISPR/Cas9* alters courtship towards red females, proving a direct link between a gene and behaviour. Our study paves the way for understanding how variation in visually-guided behaviour is encoded at the genetic level, and can contribute to adaptation and speciation.

Visual signal modulation in *Morpho* butterflies: the role of lamellar angles in the permanence of iridescent displays

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The wings of *Morpho* butterflies are covered by scales with ridges that consist of lamellae organized into a multilayer. Light reflected by the multilayers is iridescent and polarized, depending on the angle of light incidence. The organization of the wing scales can distinctly vary (Giraldo et al. 2016), and thus we hypothesized that the spatial display and degree of polarization will strongly depend on the investigated *Morpho* species.

We studied the spatial and polarization reflection profiles of the wings of males from all 30 *Morpho* species, by applying angle-dependent reflectometry, microspectrophotometry, and imaging scatterometry. We found a remarkable difference in the angular distribution of reflected light when comparing flashy and glossy *Morpho* species. The flashy species, e.g., *Morpho sulkowskyi*, reflect light into a small spatial angle with a low degree of polarization, while the reflections of glossy species, e.g., *Morpho helenor*, are wide-angled with a higher degree of polarization.

Spatial and polarization signalling will play a central role in the intraspecific communication of *Morpho* butterflies, especially during flight, when the angle of the moving wings with respect to the observer continuously changes, which is the theme of envisaged behavioral experiments.

Giraldo MA, Yoshioka S, Liu C, Stavenga DG (2016) Coloration mechanisms and phylogeny of *Morpho* butterflies. J Exp Biol 219:3936–3944.

Testing the coevolution between the diversity of iridescence and visual discrimination in *Morpho* butterflies

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Diurnal butterflies are characterized by their excellent colour vision and their colourful wing patterns. Wing colouration is often an important factor in the discrimination of sexual partners, and might be linked to the evolution of their visual system. *Morpho* butterflies have an iridescent blue colour: their appearance varies with the angles of illumination or observation. Within the *Morpho* genus, a large diversity of iridescent patterns and iridescent wing colours are observed: while some species have lost their blue iridescent phenotype, some sister-species do share strikingly similar iridescent wing colour pattern. Two sister-species in particular, *M. helenor* and *M. achilles*, live in sympatry in various countries in South America and share parallel geographic variation in wing pattern. Because wing colour pattern is frequently involved in mate choice in diurnal butterflies, we investigate whether wing coloration impacts the evolution of visual capacities in *Morpho* butterflies. By investigating genetic variations in the genes controlling the photoreceptive proteins involved in colour perception, the opsins, we detected an opsin duplication restricted to the *Morpho* species displaying a blue iridescent colouration. Using behavioural experiments in the iridescent blue species of *M. helenor*, we tested for the discrimination capacities regarding iridescence and pattern in their sexual partners. These results are the first tests of the potential coevolution between colour discrimination capacities and visual signalling in *Morpho* butterflies, and may pave the way towards identifying selective forces promoting the diversity of iridescence in *Morpho* butterflies.

Dynamic visual signals in the Common grass yellow butterflies

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Butterflies often use different visual signals to guide them in their daily task of locating nectar plants, mates, and hostplants. They can use colours, patterns and movement, or the combinations of the three, as cues, but there is a lack of understanding how different signal properties may affect perception. Using a widely studied model species, the Common grass yellow butterfly, we investigate how signals are used in different visually mediated tasks and how different signal properties might affect perception. Using visually naïve butterflies in the lab, we determined innate colour preference for nectar flower colours and further verified the result with field observations of individuals in their natural habitat. Further, utilizing the motivation of males to approach mates, we test how dynamicity in terms of moving and flashing influences signal detection in both field and lab conditions. We also plan to test how flashing influences the salience of colour signals in visually noisy environments.

How variations in light habitats reflect on visual traits and visual adaptations in three different butterfly species?

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From dark regions under canopies in woodlands to bright colourful pastures with open sky, butterflies can be spotted flying. The wide range of light intensities at which butterfly species are active likely demand to develop visual traits that would be adapted to these light conditions. For example, species from dim habitats would benefit from increasing facet diameter for maximum photon catch and therefore increase in eye size, while for species from bright habitats increasing visual acuity by increasing facet density and decreasing inter-ommatidial angle would be more valuable. In butterflies, these traits also vary along the eye regions and differ between sexes to facilitate visual motion during flight, mate detection or host-plant recognition. If and how butterfly eye properties have been adapted to different light habitats and how these might relate to differences in dispersal and behavioural requirements remains unclear.

To address this knowledge gap, we studied three temperate butterfly species from different light environments, *Pararge aegeria* (dim habitat: boreal forest, woodland and woodland borders), *Pieris napi* (mixed habitat: semi-natural grasslands and mixed forests) and *Vanessa Atalanta* (open habitat: semi-natural grasslands and forest borders). For each butterfly species, we recorded the light intensity at which each butterfly was spotted along a transect. Then we measured the visual traits including eye size, field of view, facet number and diameter, inter-ommatidial angle and eye parameter of captured butterflies. We will present the results of these analyses and discuss how light habitat may affect visually guided behaviour of these butterfly species.

Visualizing butterflies and moths: using data from a novel, high throughput, multi-spectral imaging system

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We present examples of results obtained using a novel, high-throughput multi-modal imaging system with custom built hardware and software that systematically quantifies the shapes and colors of museum specimens of butterflies and moths. Data gathered from thousands of specimens spanning the main families of butterflies and many lineages of moths provide insights into key functional differences in morphological shapes and color signaling of fore- and hindwings of males and females within and between different groups. In particular, we focus on characters associated with binocular vision, as well as those affecting flight patterns and sexual dimorphism.

Why are aposematic signals so effective but so rare?

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Evolution of animal coloration has led to multiple defence strategies, including background matching and disruptive coloration, which aim to conceal animals from predators. In contrast, warning coloration is exhibited by conspicuous animals that often possess chemical or physical defences and repel predators through aversive memory. Despite warning colours being empirically shown as an effective defence, studies on caterpillars and frog communities have found that only 5% of species are highly warning-coloured. It is commonly assumed that animals in tropical regions are more colourful, and consequently would have more warning colours. Tropical climates have long-lived predators that can retain memory of consumed toxic items and relatively consistent predation rates throughout the year, enabling effective signal learning. In contrast, predation pressure in temperate zones peaks in the spring, when naïve migratory predators and juveniles of resident species consume warning-coloured individuals at higher rates. In this study we directly test if there are more warning-coloured butterflies in the tropics when compared to subtropics and temperate areas. We collected over 1800 butterflies from 3 climate zones and will use photo analyses to assess their warning coloration. Our findings will determine if defensive strategies are climate-related and if changes in climate will make it more likely for warning-coloured species to expand their distribution range. This could increase predation pressure on palatable species and ultimately lead to their extinction. This study can aid conservation efforts by identifying which signalling strategies are most impacted by climate-related changes.

Ecology and evolution of evasive mimicry in butterflies: insights from the diverse Neotropical genus *Adelpha*

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Butterflies from the genus *Adelpha* represent an excellent example of the considerable diversity found in the Neotropical Andes. *Adelpha* diversified across a broad latitudinal and altitudinal range into around 96 species and over 130 subspecies. While *Adelpha* has been the focus of systematics, taxonomy and diversification studies, their evolution and ecology remains poorly targeted. Recent evidence supports the idea that *Adelpha*'s extreme resemblance of wing colour patterns among distantly related species is a case of convergence, although studies on the ecological role of this convergence are almost inexistent. Classical Müllerian and Batesian mimicry based in chemical defences has been hypothesized to explain this convergence. Alternatively, the hypothesis of evasive mimicry i.e., convergence of bright, conspicuous colour patterns that signal prey's ability to escape, has been proposed. We provide the first elements regarding the ecology and evolution of evasive mimicry in Neotropical butterflies such as a proof-of-concept for predator's ability to learn and generalise a visual cue associated to evasiveness of prey. In addition, we inferred the most comprehensive phylogeny for *Adelpha* and reconstructed ancestral mimicry patterns to investigate their evolution. Finally, we assessed the phylogenetic and mimicry structure of *Adelpha* communities to unravel the processes underlying species assemblages providing evidence for convergence of colour patterns, co-occurrence of co-mimics and clustering of mimicry rings by altitude. Some of the patterns observed are different from those observed in classical mimicry systems, which reflects that the mechanism underlying these two types of mimicry might be different.

Uncertain mate recognition forms social behaviours and conspicuous traits of butterflies

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Generally, intra- and inter-sexual interactions have been assumed to be qualitatively different processes. However, same-sex sexual behaviour is widespread in the animal kingdom, which may challenge this assumption. Recently, a hypothesis was proposed that regards territorial contests of butterflies as an initial phase of sexual behaviour. Inspired by this study, we investigated whether butterflies have category knowledge of "the same sex", and in light of this, we reexamined past research. We could not find any evidence that butterflies have category knowledge related to the same sex and species. Intra- and most inter-specific interactions of butterflies can be regarded as various phases of sexual behaviour. Thus, we concluded that it is sufficient to assume the category knowledge of sexual partners (that elicit some phases of mating behaviour) and of hazards (that elicit escaping) to explain butterfly interactions. For butterflies, other animals are existences that have a sexual partner element and a hazard element. We named the framework based on this assumption as partner-hazard continuum. It also provides a reasonable explanation for evolution of sex- and species-specific traits of butterflies, which generally appeared on males. Based on the signal detection theory, any male traits advertising "sexual availability" to females should increase the probability that males will be accepted by conspecific females because such traits increase females' discriminability. That is, these traits enable females to reduce the risk of incorrect acceptance of other species including predators.

Anti-predator defences of neotropical Skippers (Hesperiidae), more complex than previously thought

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Prey organisms are under constant selective pressure by various predators, thus, multiple strategies have evolved to evade, to confuse or to mislead predator attacks. Skippers (Hesperiidae) are diurnal butterflies long suspected to utilise different strategies to evade visual predators (e.g., insectivorous birds). For example, they have one of the fastest startle reflexes in the animal kingdom, and their highly erratic flight may be advertised to avian predators by their external phenotype. By using a laboratory protocol, we found that hindwing tails in artificially escaping, dummy Skippers can be learnt by a surrogate predator, thus, showing 'butterfly tails' can be effective morphological cues to advertise evasiveness, in addition to their role in deflecting predator attacks. In field experiments in the Peruvian Andes, we have uncovered an additional defence strategy in Skippers; some species are unpalatable for domestic chicks (a common surrogate model for testing butterfly palatability). This suggests that Skippers have multimodal defences, being highly evasive and highly unpalatable as other aposematic neotropical butterflies such as *Heliconius* or Ithomiini. Finally, by conducting aviary experiments across seasons and habitats using local insectivorous birds, we have gathered evidence suggesting that such predators are aware of the defences and significantly reduced their attack rate to Skippers compared to control palatable and slow butterflies. Altogether, by using a combination of lab and field experiments, we are shedding light upon the possible drivers explaining multimodal defences in Skipper butterflies.

Butterfly diversity under climate change

KEYNOTE TALK

Range shifts of butterflies from past to future

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Climate change has a worldwide impact on biodiversity and ecosystem functions, in particular by causing shifts in species distributions and changes in species communities. In various studies we analyse altitudinal range shifts of mountain and lowland butterfly species and burnet moth species over the past 7 decades across the federal state of Salzburg (northern Austria) spanning an altitudinal gradient of more than 2500m. For each species, we compiled species-specific traits on their ecology, behaviour, and life-cycle. During the last seven decades the butterflies have shifted their average occurrence and also lower and upper occurrence limits more than 300 metres uphill. This shift is particularly obvious for the last 10 years. Our results underline that the effects of climate warming have a strong and currently increasing impact on the patterns of species distribution and local community compositions and biodiversity. Apparently, ubiquitous, mobile species with a broad ecological amplitude can cope better with environmental changes than specialist and sedentary species.

Predicting sudden and widespread biodiversity loss on a rapidly warming planet: when and where does biology change things?

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As our planet rapidly warms, risks to biodiversity continue to escalate. Existing models indicate that rising temperatures will lead to an abrupt expansion in the number of populations exposed to dangerous climatic conditions, and that entire communities of species may be at risk of precipitous collapse as thresholds of warming are exceeded. A major simplification of these models, however, is that they do not consider the biology of organisms, such as life history and ecology, that may mediate differential responses to climate change. To study how organismal biology may dampen, delay or amplify risks of abrupt community collapses, we used a rich dataset of butterfly abundance from the mountains of central Spain that spans from 1980 to the present. Here, many of the focal species are at the hot edge of their range, with some already exhibiting climate-associated declines, range contractions and temperature induced phenological shifts. By combining abundance data with cutting-edge models of historic microclimate, we show that species abundance temperature curves (a proxy for the thermal niche) are clustered, suggesting high threats of tipping points in butterfly communities in this system. In addition, we investigate how morphological, behavioural and physiological traits mediate clustering of tipping points in communities.

Long-term datasets reveal climatic nuance in moth responses to global change

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Insects are sensitive to global change. Yet the mechanisms underpinning responses to warming and changing rainfall patterns are often non-linear and taxon-specific. We used two novel datasets collected by a single observer throughout the 1990s to today to investigate: 1) how moth occurrence has changed over years, 2) if disturbance (e.g., fire) and seasonal weather patterns have impacted moth populations, and 3) whether syndromic patterns emerge in responses to suites of climatic changes. Our study focuses in northern California within two areas: Davis, a low elevation, suburban community bordered by agricultural land and Stebbins Cold Canyon Nature Preserve, a relatively undeveloped site located at a higher elevation.

Our investigation of 86 species in Davis and 170 species in Cold Canyon emphasizes the importance of a nuanced understanding of the impact of climate change on Lepidoptera. Effects of climate on moth occurrences were strong and varied considerably within and across sites. Importantly, we often observed opposite effects of seasonal high and low temperatures. At the suburban site, increasing spring night-time temperatures strongly drove moth declines whereas increasing spring daytime temperatures elevated moth incidence. In the more natural site, rising fall temperatures imposed the strongest negative influence on moth occurrence. Years since fire strongly increased moth occurrence, suggesting that moth populations are negatively affected by fire but can recover over time. Our results point to a future for moths in the biodiversity hotspot of northern California that is defined by seasonal climatic shifts and by increasingly common and severe fires regionally.

Three ladies and an admiral: exploring *Vanessa* response to climate change across multiple decades and a steep elevational gradient

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Following the charge to better understand the effects of a changing climate on insect population trajectories, various studies have implicated facets of climate that are important in driving either (a) population trends of insect fauna assessed at a regional or broader scales, or (b) population trends for individual insect species, often of conservation concern. That taxonomic resolution, at broad or very narrow scales, also characterizes work centering on butterflies, wherein the potential for among-species, intra-genus heterogeneity in response to the same climatic cues is rarely in detail. Here, using data from a set of ten permanent, long-term (between 35 and 50 years) monitoring transects established across Northern California in the United States, we assessed which aspects of weather were most important for predicting presence during the flight season of four species belonging to the *Vanessa* genus: *V. annabella*, *V. atalanta*, *V. cardui*, and *V. virginiensis*. Climate data were sourced from the TerraClimate platform, and an additional variable, climate departure as a multivariate measure of climate change, was generated by calculating departure from baseline conditions for precipitation and temperature for the years 1958–1985. Our results thus far suggest that climate departure is one of the most important aspects of weather in predicting presence during the flight season for all four species; the effect is negative for three species but positive for *V. cardui*. Other heterogeneity in weather effects will be discussed and interpreted in the context of natural history variation (including migratory behavior), and stability or decline of each species.

Zoogeographical changes in the Israeli butterflies during the last 33 years indicate climate change trends in the Middle East

Dubi Benyamini

4D MicroRobotics, Bet Arye, Israel & President, The Israeli Lepidopterist's Society

My presentation compare the zoogeographical composition change over the last 33 years that appear in my Israeli field guides 1990–2022.

The calculation of butterfly zoogeographical composition changes is an easy and fast way to present distribution changes caused by the climate change compared with the hard work of analyzing tens of thousands of monitoring records.

Israel is a unique crossroads of several Zoogeographical Regions located among three continents – Europe, Asia & Africa. It is the only existing active land-bridge between the Palaearctic & Afrotropical regions;

- The Palaearctic Region in the North with its southern ecotone is located north of Be'er Sheva – in the northern Negev Desert.
- The Eremic Zone; the eastern extension of the Saharan Desert in the south with its northern Ecotone is located south of Beer Sheva.
- The Afrotropical Region's Sudanian penetration along the Rift Valley is located in the Arava & Jordan Valley
- Three Irano-Turanian sub-regions exist: Mt Hermon (N Israel), the Irano-Turanian eastern zone and the southern Irano-Turanian zone of the central Negev mountains.

The Israeli butterfly fauna is composed of butterflies from these zoogeographical regions and the active land bridge among three continents is a unique migration corridor for butterflies flying usually northwards in the spring and southwards in the autumn. Global warming is reflected in changes of the said ecotones where the general trend is northwards expansion of the Eremic & Afrotropical butterflies while the Palaearctic species are usually in retreat.

What drives (meta)population dynamics of the bog fritillary butterfly? The relative importance of habitat quality, parasitism, and climate

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The bog fritillary (*Boloria eunomia*) is a specialist butterfly inhabiting wet meadows and peat bogs, originally chosen as a model system to study metapopulation dynamics because, for this species, the landscape can be easily defined by a clearcut but meaningful “habitat vs matrix” dichotomy. It has been intensively studied in Belgium since 1992, becoming a landmark study system for (meta)population dynamics and viability. Here, we show how we refined our understanding of its population dynamics, in its density-dependent and density-independent aspects, by adding levels of detail in the quantification of its habitat quality, as well as considering the impact of parasitism and climate. We replaced the simplified black and white view (“habitat quality is constant, only habitat size and configuration in the matrix matters”) by a more detailed “shades of grey” view (“habitat quality varies in space and time according to resource availability”). We present how we synthesized data collected on the species over 30 years to reconstruct spatiotemporal changes in habitat quality, filling data gaps via interpolation, proxy variables or expert assessment. These habitat quality data, combined with relevant climatic parameters covering each life stage of the butterfly, were used to revisit what drives population growth rate of the bog fritillary. This confirmed the strong density-dependence previously observed, uncovered strong influence of parasitism as well as climate during larval stages, but did not indicate a direct effect of habitat quality. Eventually, we discuss the consequences for species viability and conservation under climate change.

Eco–evo–devo of lepidopteran color patterns

Convergence in sympatric swallowtail butterflies reveals ecological interactions as a key driver of worldwide trait diversification

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Ecological interactions may fuel phenotypic diversification in sympatric species. While competition can enhance trait divergence, other ecological interactions may promote convergence in sympatric species. Within butterflies, evolutionary convergences in wing color patterns have been reported between distantly-related species, especially in females from palatable species, where mimetic color patterns are promoted by predator communities shared with defended species living in sympatry. Wing color patterns are also often involved in species recognition in butterflies, and divergence in this trait has been reported in closely-related species living in sympatry as a result of reproductive character displacement. Here, we investigate the effect of sympatry between species on the convergence vs. divergence of their wing color patterns in relation to phylogenetic distance, focusing on the iconic swallowtail butterflies (family Papilionidae). We developed a new unsupervised machine-learning-based method to estimate phenotypic distances among wing color patterns from 337 species allowing to finely quantify morphological diversity at global scale within and among species, allowing to compute pairwise phenotypic distances between sympatric and allopatric species pairs. We found that sympatry promoted strong convergence, while divergence in sympatry was less frequent and weaker. The effect of sympatry on convergence was stronger on females than males, suggesting that differential selective pressures acting on the two sexes drove sexual dimorphism. Our results highlight the significant effect of ecological interactions driven by predation pressures on trait diversification in Papilionidae and evidence the interaction between phylogenetic proximity and ecological interactions in sympatry acting on macroevolutionary patterns of phenotypic diversification.

False head: evolution of another head in butterflies

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Many butterflies, mainly lycaenids and riodinids, possess a complex set of characters at the posterior hindwing end, superficially resembling their head. This ‘false head’ (FH) has been hypothesised to deflect predator attacks towards the FH area, which some studies support. However, many hypotheses regarding the mechanism of how FH deters predator attacks remain to be tested. The presence of the many traits constituting a false head varies across butterflies, and a clear understanding of the diversity and evolution of FH traits across butterflies is lacking. We hypothesised that FH traits evolved from simple to complex, more elaborate, traits in a correlated manner. Such a pattern would imply correlated evolution of traits through common selective forces. Using a phylogenetic framework with >900 lycaenid species, our results illustrate patterns of evolution of five FH traits – (i) false eyespots, (ii) conspicuous colouration in the FH area, (iii) false antennae, (iv) head-like contour of the posterior hindwing, and (v) convergent lines – across the phylogeny. The evolution of convergent lines – continuous lines from the forewing to the hindwing that converge and meet at the FH area – was highly dependent on phylogeny and found only in Theclinae and one Aphnaeinae genus. The evolution of the remaining four FH traits was correlated across the phylogeny, with the correlation between false eyespots and false antennae being the strongest. Thus, our findings suggest a functional association among these false head traits. Our analyses also shed light on the order of evolution of these traits across the phylogeny.

Does growth rate influence eyespot size? – A comparative study of three sympatric satyrine butterflies

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Growth rate is an important life history trait that can have strong consequences for the fitness of an organism. In butterflies, eyespots are colour patterns on wings that help in antipredator defence as well as in sexual signaling and are known to be phenotypically plastic. Previous studies have shown that in satyrine butterflies, eyespot development is positively correlated with temperature, and it is also known that temperature regulates growth rate. These results, along with results from other studies in butterflies, suggest a role of growth rate in determination of eyespot size. We hypothesized that eyespot development is positively correlated with growth rate. We tested this in three sympatric satyrine butterflies (*Mycalesis mineus*, *Ypthima huebneri*, and *Melanitis leda*) through manipulations of growth rate by varying temperature and host plants. We measured the relative forewing and hindwing eyespot sizes and their correlations with temperature, host plant, and life-history traits. Our results showed that there was a broad positive correlation between growth rate and eyespot size; however, it was species and sex-specific and also varied with temperature and eyespot position on the wing. Eyespot size also generally correlated with larval duration, with some exceptions. Our results show that selection and regulation of eyespot development may differ across closely related species, and within species across sexes and eyespot positions.

A meta-analysis of butterfly structural colors: color range and phylogenetic distribution

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Butterfly scales are among the richest natural sources of optical nanostructures, which produce structural color and iridescence. Over the past century, several recurring nanostructure types have been described, including ridge multilayers, lumen gyroids, and lower lamina thin films. While the optical mechanisms of these nanostructure classes are known, their phylogenetic distributions and functional ranges have not been described in detail. Here, we share a database of color and morphology data for more than 350 previously published butterfly structural colors. Using our database, we conduct a meta-analysis to assess the prevalence, phylogenetic distribution, and color range of each type of nanostructure. Butterfly structural colors are ubiquitous in short wavelengths but extremely rare in long wavelengths, especially red. In particular, blue (around 450 nm) occurs in more lineages and is produced by more kinds of nanostructure than other hues. Nanostructure categories differ in color range, brightness, phylogenetic distribution, and frequency. For example, lamina thin films are least bright; perforated multilayers have been reported the most often but are almost entirely restricted to the family Lycaenidae; and 3-dimensional photonic crystals, including gyroids, have the narrowest wavelength range (from about 450 to 550 nm). We interpret and discuss these findings in the context of nanostructure evolution, with reference to convergence, constraint, functional tradeoffs, and relationships with pigmentary color.

The actin cytoskeleton plays multiple roles in structural colour formation in butterfly wing scales

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Vivid structural colours in butterflies are produced by the interaction of light with tiny nanostructures within the wing scales. Despite the diversity and evolutionary significance of these colours, the developmental processes controlling the construction of optically-precise nanostructures remain largely unknown. This is particularly the case for multilayer reflectors within some butterfly scales, which form through modification of cuticle ridges into a stack of reflective layers. Within developing scales, the actin cytoskeleton controls overall cell shape and positioning of the cuticle ridges and it has been suggested to be involved in sculpting nanostructures. Here, we use super-resolution imaging techniques and perturbation experiments to uncover novel roles of the actin cytoskeleton in wing scale and nanostructure development in the butterfly *Heliconius sara*. Through comparative analyses of iridescent and non-iridescent scales at both developmental and adult stages we demonstrate a role of actin in maximising the density of light reflecting ridges. Then we interrogate the developing scales with TauSTED super-resolution microscopy to reveal a novel branched network of actin which continually reorganises during nanostructure development. Finally, chemical perturbation of actin during nanostructure development disrupts the formation of the multilayer reflectors, leading to loss of structural colour in adult butterflies. Overall, we demonstrate actin plays vital templating roles during structural color formation in iridescent butterfly scales, through mechanisms which may be universal to controlling ridge and nanostructure architecture in all lepidoptera.

Developmental differences between iridescent and non-iridescent scale nanostructures in *Heliconius sara* butterflies

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Butterfly wings are patterned in a mosaic-like manner by arrays of individual units called scales that are the apical projections of single cells. The scale's cuticular scaffold is a composite material composed of chitin embedded in a protein matrix. Each scale has one colour determined by the deposition of pigments in the scale cuticle and/or by the development of nanostructures in the cuticle that produce structural colours. How single cells control the development of intricately patterned nanostructures remains unknown. To address the development of butterfly scale nanostructures, we compared iridescent, structurally coloured blue scales in *Heliconius sara* butterflies with non-iridescent black scales. Using a developmental time series of electron microscopy images, we identified the important stages and differences in nanostructure formation between the two scale types. Further, using RNA-seq comparisons between the iridescent and non-iridescent tissues at various time points, we identified a membrane anchored matrix protein important for differential regulation of nanostructure formation.

Cytoskeletal Contributions to Scale Shape and the Evolution of Wing Transparency

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Butterfly and moth wings are adorned with hundreds of thousands of scales—macroscopic polysaccharide husks of elaborate geometry secreted by individual cells in the pupal stage. The morphologies of these scales are tightly regulated to the nanometer and confer functional advantages to the insect, namely in the realms of coloration, thermoregulation, and aerodynamics. The clear link between form and function within a single cell therefore begs an opportunity to merge development, cell biology, and evolution to understand motifs and principles of biological design. To build a comparative framework of these structures, visualizing cellular organization across many species is essential. As such, we have leveraged live cell dyes with an assortment of novel manipulations and high-resolution microscopy to visualize dynamics of nuclei, cytoskeletal proteins, and other cellular components live in developing insect wings. As a demonstration of the strength of this approach, we provide a framework for how early organization of actin filaments and microtubules coordinate proper planar-polarized growth across intra- and interspecific variation in lepidopteran scale morphologies. In particular, we home in on the ecological phenomenon of wing transparency wherein scales can, among other strategies, be modified from flat lamelliform projections to cylindrical bristles.

Elucidating the development of structural colour in butterflies using hyperspectral microscopy

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Structural or physical colour is a fascinating phenomenon produced by the interference and scattering of light on nanostructures. In butterflies, chitinous nanostructures of varied complexity are responsible for virtually all vibrant blue and green colours. A particularly remarkable case are the green scales of *Erora opisena*. The scales contain separated photonic crystallites comprising a labyrinthic network with a single Gyroid geometry. It is hypothesized that the crystallites are templated during scale development by the folding of an intracellular organelle membrane, however it is not yet possible to spatially resolve their formation with *in vivo* imaging methods. Here, we present a wide field hyperspectral microscopy technique which allows fast (~3 secs) reflection and transmission measurements of single butterfly wing scales, and reflection measurements from wing patches. While wide field light microscopy cannot spatially resolve the nanostructure itself, the geometry of individual crystallites can be inferred from the measured spectra. We demonstrate the potential of this technique for *in vivo* imaging by measuring weaker optical signals from adult wing scales immersed in refractive index matching fluids, simulating the environment of developing wing scales. This raises the hope that hyperspectral microscopy could be applied to a developing pupa, gaining insight into the structure formation processes *in vivo*.

Speculations on specularity: gold and bronze to moths, silver to butterflies

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The interplay of pigmentary and structural colouration of wing scales determines the visual appearance of butterflies and moths. All Lepidoptera seem to share the same ultrastructural elements and photonic mechanisms for colouration, but scientific focus has been mostly on diurnal butterflies, likely due to their easier identification, bigger colour gamut and general appeal.

In the domain of specular (directional or "metallic") colouration, blue and green that arise from interference of light with multilayers and photonic crystal structures, are often narrow band, and may be strong visual signals, particularly in diurnal butterflies. Broad-band specular colours (e.g., gold) are achieved by combining pigmentary absorption, thin-film effects and layering of different types of scales. The perforation of the upper lamina of the covering scales is the main control mechanism of broad band specularity.

Here, we present an overview of specular broad band colours across Lepidoptera. Butterflies often exhibit silver spots (e.g., nymphalids *Argynnis*, *Dione*, *Agraulis*) and semi-specular colouration is observed as silver-white (lycaenid sunbeams, *Curetis*). Meanwhile, gold (specular yellow) and bronze (specular red/brown) can be found on moths. We show that in the noctuid moth *Diachrysia chryson*, the light reflected from gold and bronze patches on the upper forewings forms a line-shaped spatial beacon. In the gold scales, UV-blue light is likely being absorbed by a pigment absorbing maximally at ~380 nm.

Finally, we speculate on the importance of the photic environment for signalling and crypsis using broad band colours, and on the curious rarity of butterfly gold.

Transcription factor *bric-a-brac* regulates known pigmentation, patterning and scale structure genes across Lepidoptera

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The transcription factor-encoding gene *bric-a-brac* (*bab*) is responsible for body pigmentation in many *Drosophila* species. A recent study in sulphur butterflies, has shown that *bab* regulates UV-iridescent wing scale morphology. We explore the role of *bab* as a regulator of wing color pattern across Lepidoptera. Using CRISPR-cas9 genome editing, we knock out *bab* in three different nymphalid butterflies and one species of moth. We observe that *bab* is responsible for development of proper scale structure across Lepidoptera. Not only that, *bab* knockouts (KOs) across species showed increased deposition of pigments in scale cells in wings and bodies transcending known pattern boundaries. Besides hyperpigmentation, *bab* KOs in *Junonia coenia* show enhanced eyespot ring patterns and, in *Heliconious erato* switches white patterns to yellow and spreads red patterns across previously established pattern boundaries. With the help of *bab* ChIP-seq, in multiple species across different families of butterflies, we establish direct relationship of *bab* with several downstream patterning genes and compare how these binding sites and targets have evolved along the butterfly lineage. Differential gene expression results in *bab* KOs when overlapped with direct *bab* targets also helps us establish specific regulatory relationship of *bab* with some downstream targets already implicated in regulating wing pattern, pigmentation, and scale structures. Therefore, we use a many pronged approaches to understand how a transcription factor can regulate previously known butterfly wing patterning regulators and how this interaction has changed across evolutionary time.

Deciphering the Evolution of Sex-Limited Butterfly Wing Color Polymorphisms

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The study of discrete polymorphisms has significantly advanced our understanding of the genetic basis and evolutionary origins of novel phenotypic traits. In particular, butterfly wing coloration offers a rich array of diverse polymorphisms, suitable for identifying causal mutations and investigating allele functionality within shared genomic backgrounds. Intriguingly, wing color polymorphisms are predominantly either shared between both sexes or limited to females, posing questions on the evolution of sex-limited traits in butterflies.

Here we will contrast two case studies of female-limited wing color polymorphisms, highlighting the different genetic bases and implications for the evolution of sex-limited traits in butterflies. In the first case study, we explore a regionally isolated female-limited color morph, while the second case study delves into an ancient polymorphism shared across multiple species within a genus.

By contrasting these two cases with historical and recent literature, we aim to provide insights into the factors driving the prevalence of female-limited polymorphisms and the evolutionary forces shaping sex-limited traits in butterflies. Our findings contribute to the broader understanding of the ecological, evolutionary, and developmental aspects of butterfly coloration and offer a valuable foundation for future research on sex-limited trait evolution across diverse taxa.

Genetics of a mimicry ring switch in ithomiine butterflies

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Increasing our knowledge of genetic architecture and developmental processes causing phenotypic differences between organisms aids our understanding of how evolution gives rise to the huge diversity found in nature. Diversity in certain groups of toxic butterflies, among others ithomiine butterflies, can be directly linked to selective forces shaped by predation and aposematism where different co-localizing species are known to mimic each other, forming so-called mimicry rings of similarly looking species. These mimicry rings are an excellent system to study phenotypic change and the convergent evolution of phenotypic change, since there often exist several subspecies with different wing colour patterns within one species, and different species that exhibit convergent changes in phenotypes. This work looks at the genes and genetic architecture underlying convergent evolution of the presence of a yellow band in *Mechanitis* and *Melinaea* from Ecuador and Colombia to elucidate the genomic changes that control morphological variation and diversification. A genome-wide association study identified that a regulatory region of *cortex*, a known colour pattern gene in Lepidoptera, plays a role in the yellow band pattern in *Mechanitis* butterflies. These results have been functionally validated through genetic modification using CRISPR-Cas9, and the expression pattern of this gene are investigated through antibody staining, in situ hybridization and RNAseq.

Transposable element insertions are associated with Batesian mimicry in the pantropical butterfly *Hypolimnas misippus*

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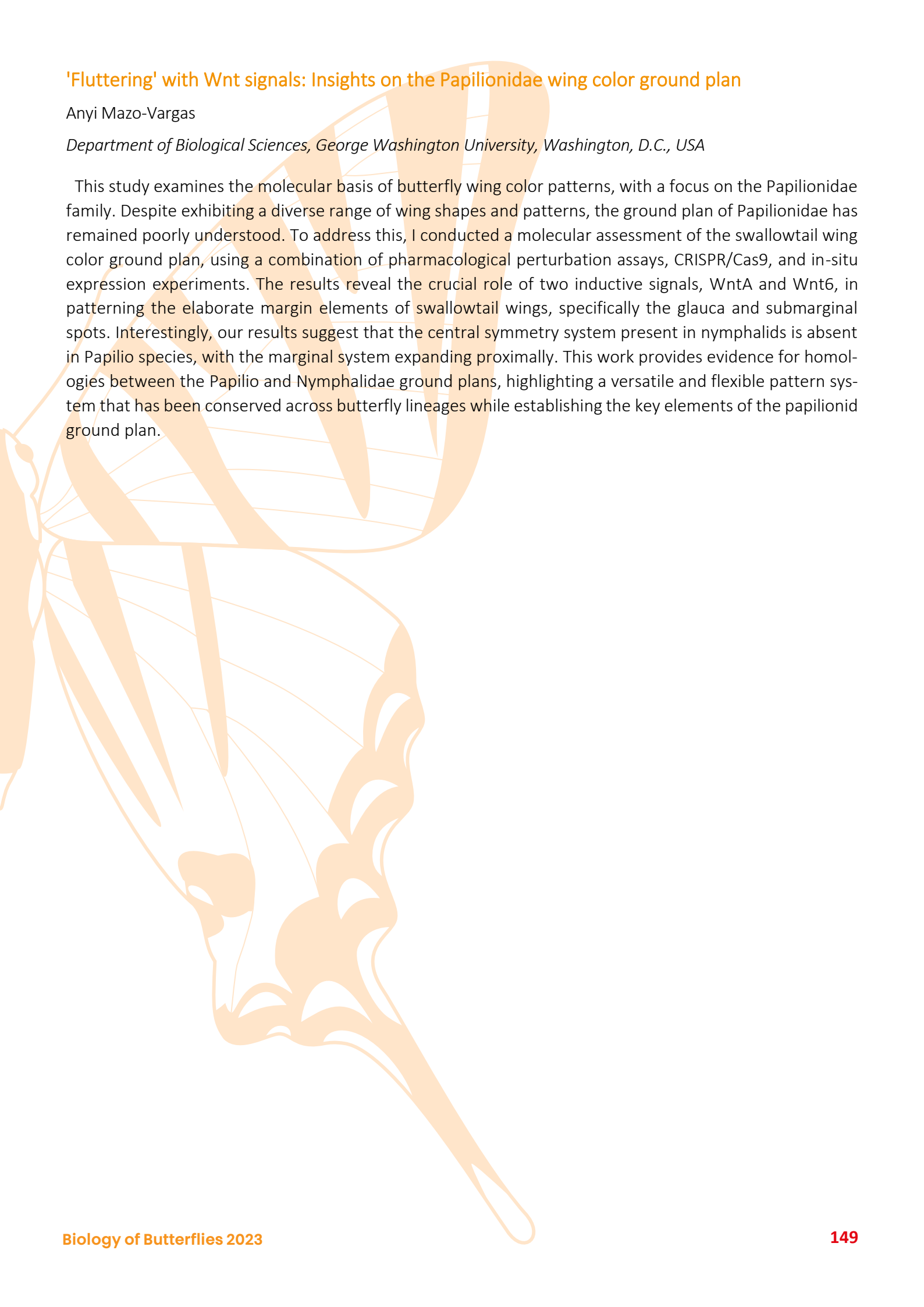
Atavism, or evolutionary reversal to an ancestral character, is a rare event that often has maladaptive effects because of the mismatch between the trait and the environment. *Hypolimnas* are a diverse genus of butterflies that show several instances of wing Batesian mimicry, resembling multiple, morphologically distinct, model species. Here we present an example of adaptive atavism and convergent evolution in *Hypolimnas misippus*, in which the morphological reversal to the ancestral character via regulatory rewiring results in a new instance of Batesian mimicry. In *H. misippus*, mimicry is limited to females, which are polymorphic showing detailed resemblances to the four morphs of *Danaus chrysippus* and are maintained as a stable polymorphism with little correlation to local model frequencies. Using haplotagging, a linked-read sequencing technology, we show that two large insertions are found at the non-coding locus associated with mimicry in *H. misippus* and validate these using molecular methods. We demonstrate that the insertions are formed by several transposable elements (TE) and are found in the dominant allele which produces the ancestral and mimetic phenotype. Through comparative analysis with additional *Hypolimnas* species, we show that the dominant allele is derived, suggesting that the TEs disrupt a cis-regulatory element that causes the reversion to an ancestral character that is re-used for Batesian mimicry of a new model species. Our results present a complex example of convergent evolution and adaptive atavism, in which the same pattern element has evolved independently multiple times and is repeatedly involved in Batesian mimicry of distinct model species.

Spatial and temporal regulation of Wnt signaling pathway members in the development of butterfly eyespots

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Wnt signaling is essential in the differentiation of eyespot color patterns on the wings of *Bicyclus anynana* butterflies, however, the expression and function of most of the Wnt pathway members, as well as their interactions, remain unknown. In the present work, we use immunofluorescence and hybridization chain reaction to explore the genes involved in Wnt signaling in a spatial-temporal context in the larval and pupal wings of this species. We examined the signal transducer Armadillo (Arm), all seven *Wnt* ligands, and four *frizzled* receptors present in the genome. We then used CRISPR-Cas9 to test the function of *arm* and *frizzled4* and discovered a clear link between the canonical (Arm/ β -catenin dependent) and non-canonical (Arm/ β -catenin independent) Wnt signaling in the differentiation of the eyespot centers during the larval wing development. In the later pupal stage, we propose that different Wnt signaling members are likely interacting to control the active domains of each other to pattern the colored rings.



'Fluttering' with Wnt signals: Insights on the Papilionidae wing color ground plan

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This study examines the molecular basis of butterfly wing color patterns, with a focus on the Papilionidae family. Despite exhibiting a diverse range of wing shapes and patterns, the ground plan of Papilionidae has remained poorly understood. To address this, I conducted a molecular assessment of the swallowtail wing color ground plan, using a combination of pharmacological perturbation assays, CRISPR/Cas9, and in-situ expression experiments. The results reveal the crucial role of two inductive signals, WntA and Wnt6, in patterning the elaborate margin elements of swallowtail wings, specifically the glauca and submarginal spots. Interestingly, our results suggest that the central symmetry system present in nymphalids is absent in Papilio species, with the marginal system expanding proximally. This work provides evidence for homologies between the Papilio and Nymphalidae ground plans, highlighting a versatile and flexible pattern system that has been conserved across butterfly lineages while establishing the key elements of the papilionid ground plan.

Novel traits that emerge from the co-option of pre-existing gene regulatory networks make widespread use of pleiotropic enhancers

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Novel traits often emerge from the redeployment of old genes to a novel location in the body, and this process can leave behind distinct genomic signatures. While there is ample support in the literature that new enhancers often accompany a gene's novel expression domain, the reuse of pre-existent enhancers is predicted to happen when large gene regulatory networks, rather than single genes, get co-opted to a novel location. Butterfly eyespots are a complex novel trait recently proposed to have emerged from the co-option of the appendage gene regulatory network to the wing. Here, combining open chromatin assay and genome editing, we identify a series of enhancers in two genes, *Distal-less* and *Spalt*, that, when disrupted, lead to disruptions in eyespots, antennae, legs, and wings, making them essential enhancers for the development of eyespots as well as ventral and dorsal limbs, and thus, highly pleiotropic. Our study shows that novel traits that result from the co-option of large gene networks contain genes with pleiotropic enhancers and also shows that enhancer modularity is not a defining character of all enhancers.

Population, landscape, and conservation genetics of butterflies

KEYNOTE TALK

Museomics to retrace the decline of butterfly populations throughout the 20th century

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The large-scale decline of insects in the past decades starts to be widely documented. The mechanisms, the kinetic and the causes of this decline, and in particular the role of anthropogenic activities, remain poorly understood. Indeed, studies documenting this decline are based on recent trends, since the 1970s, and rely on biomass estimations that do not fully reflect the state of populations. However, samples are available to undertake a large-scale study, both spatially and temporally (throughout the 20th century). The museum collections contain insects collected throughout this period and form real time series. Thanks to recent developments in museomics, it is now possible to access the genetic information carried by these samples and thus to study their demographic evolution over time. Through two studies, we will show that museum collection specimens constitute an invaluable sample source, potentially providing direct access to genetic variation over time. The first study used large time series of two butterfly species in Finland, *Erebia embla* and *Lycaena helle*, and showed that despite local variations, overall genetic erosion has increased across the last hundred years, as revealed by signatures of allele extinction and heterozygosity decrease. In one of the two butterflies, isolation by distance also increased through time, suggesting an increase in habitat fragmentation. The second study is a large study across Switzerland including two common butterfly species, *Polyommatus icarus* and *Pieris napi*, and aims to track specific responses to global changes.

Role of genetics in impacting population persistence under variable environments

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The role of genetic variation in population persistence has gained renewed interest over the last few years as genomes from critically endangered or even extinct species are being sequenced. Understanding the value of neutral, adaptive and/or deleterious genetic variation in increasing population persistence under changing environmental conditions is one of the key challenges in conservation biology. We have used the Glanville fritillary (*Melitaea cinxia*) butterfly and its metapopulation in the Åland islands, SW Finland as a study system over two decades to assess how genetic variation impacts local population dynamics and persistence. In this talk I will give an overview of some of the key results. In particular, I will highlight the role of adaptive genetic variation in dispersal and the role of neutral genetic variation in influencing fitness, population performance and extinction, and how these effects interact with demography and environmental variables. Overall, our results suggests that conclusions about the importance of genetic variation for predicting fitness and extinction risk in wild spatially structured populations requires careful consideration of the complex interplay between genetic, environment, and demographic factors in nature.

Human and climatic impact on four emblematic species of butterflies in Franche-Comté and implications for conservation management

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Peat bogs butterflies are characterized by their high level of habitat specialization, depending on peatlands complex hydrological functioning and natural fragmented distribution. Many of these species are declining and protected in Europe. To inform conservation measures in the context of climate and land-use change, it is essential to identify the importance of each of these factors in population declines. The Jura massif is the last French area where the Large heath (*Coenonympha tullia*), the Violet Copper (*Lycaena helle*), the Scarce heath (*Coenonympha hero*) and the Cranberry fritillary (*Boloria aquilonaris*), are co-distributed. Based on a non-lethal sampling of local populations, we used SNP markers and SDMs to study their demographic responses to past climate changes since the Last Interglacial to current days. We showed that despite important degradation of climatic conditions between the Last Glacial Maximum and Holocene, they have succeeded in limiting their ancestral population contraction. Besides, the dramatic decline they are currently experiencing corresponds to an improvement in the climatic conditions these species require, therefore supporting the hypothesis that habitat and landscape connectivity degradation is the main driver of population decline. We now study each species landscape connectivity within the massif to identify features influencing gene flow, using both Mixed Linear Population-Effect models and graph theory. The impact of the restoration or loss of habitat patches and ecological corridors on genetic diversity is also assessed in specific sectors using genetic simulations. All results are discussed with local field experts to propose specific management measures for each species.

Does the human altered landscape isolate butterfly populations?

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Changes in land use and composition form the largest factor contributing to global biodiversity decline. As populations decline, genetic diversity is also expected to decrease due to the increasing force of genetic drift and increase in rates of inbreeding, resulting in a loss of adaptive potential and unmasking of deleterious genetic load reinforcing the environmental factors driving decline. As populations become more isolated, the chance for gene flow to rescue these at-risk populations with new genetic variation decreases, making maintenance of functional connectivity necessary for population persistence. In this study, we combine whole genomic data with land usage data to estimate the effects of arable, forest, and grassland areas on the genetic diversity and functional connectivity of three butterfly species. These species vary in their level of specialization and are expected to have different sensitivities to these landscapes. We observe a generally positive effect of grassland area on genetic diversity, but different effects of arable and forest land on the generalist and specialist species. We identify strong genetic isolation in the specialist species, compared to the near absence of differentiation in the generalist. Finally, we observe that genetic diversity is directly correlated with recent inbreeding rates in the specialist, *Cyaniris semiargus*, suggesting that its threat status may be underestimated, and restoration of functional connectivity is necessary for its conservation. These results demonstrate that grasslands play an important role in maintaining butterfly genetic diversity, and that genetic data can reveal necessary conservation actions that may go unnoticed with census data alone.

Population genetics and rangewide dynamics of the threatened prairie butterfly, *Hesperia dacotae*

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The Dakota Skipper (*Hesperia dacotae*) is a prairie specialist in the midwestern United States and Canada. The focus of an active international and multi-agency recovery program, the Dakota skipper currently receives federal protection from both countries. To evaluate local and landscape-level genetic diversity, we performed a full-genome Illumina scan and developed seven polymorphic microsatellite markers for genotyping across the entire species range. We also screened all field-sampled individuals for the presence of the bacteria *Wolbachia* to inform captive breeding efforts and reintroduction plans (as *Wolbachia* may have negative population-level impacts). Molecular results show relatively high levels of genetic diversity and relatively low genetic differentiation between populations across the range. As these findings do not match expectations given the species low dispersal capabilities and the extent of habitat fragmentation, we propose three possible explanations and their conservation implications: 1) extant populations are large enough to prevent genetic drift; 2) extant populations maintain gene flow through stepping stone populations; and 3) there is a time lag between the response of the genetic markers used and the potential population fragmentation. An isolated instance of *Wolbachia* indicates that the bacteria is not likely a major concern at this time in this species, but that regular monitoring of *Wolbachia* may be useful to detect any changes in infection frequency.

Allopatric and ecological diversification in *Cyaniris semiargus* (Lepidoptera, Lycaenidae)

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Cyaniris semiargus, the Mazarine blue, is a relatively common Lycaenidae in the western Palaearctic, typically inhabiting grasslands and feeding on various Fabaceae species. It is widely distributed across most of its range but has somewhat isolated populations at mountain ranges at the southern part of its range. Previous studies on mtDNA revealed three lineages in southern isolates, one of which also individually represents one of two evolutionary significant units and corresponds to a population from Doñana, southwestern Spain. This population represents an ecological contradiction: it occupies sandy areas at or slightly above sea level and the larvae feed exclusively on *Armeria velutina* (Plumbaginaceae). Another population with the same apparent ecological needs was later found in Aljezur, southwestern Portugal. Our aim was to explore these lineages and patterns of differentiation with ddRADseq data. Results suggest the existence of two different lineages in the southern isolated populations and very low levels of admixture between these and the widely distributed main lineage. Results also indicate mild to strong divergence of each southern isolate. Further analyses on gene flow, species delimitation, ecology and morphology need to be done, but we have solid indication that these populations present unique diversity important to consider in terms of conservation.

Phylogeography and conservation of *Melitaea diamina* (Lepidoptera, Nymphalidae): southern relict populations trapped by climate change

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Melitaea diamina, commonly known as the false heath fritillary, is a butterfly species found mainly in temperate areas of Europe and Asia. The species has a patchy distribution, and its populations, especially the southernmost ones, are often scattered on mountain areas and isolated from one another. This suggests that they represent relicts of the species' more extensive range during the last cold phase. Previous studies using mitochondrial DNA data indicated the presence of several lineages in Europe, with the notable differentiation of certain geographically isolated Iberian populations. In this study, we investigated European populations of *M. diamina* in order to unravel the origin of the multiple Iberian lineages and find possible explanations. To achieve the desired level of detail, we generated a ddRADseq dataset, which we subsequently used to perform various analyses, ranging from phylogenomics to model and non-model based population genomics, and demographic analyses. The results follow a pattern similar to what has been found in other Lepidoptera species, showing limited genetic diversity across the majority of the species' range, but with a gradual increase towards the Iberian Peninsula. The analyses indicated the presence of at least three different lineages within the peninsula, with minimal genetic admixture between them. Some of these lineages correspond to genetically unique and geographically isolated populations, that are restricted high-altitude areas and therefore would be threatened by further temperature raise brought by climate change. These findings highlight the importance of preserving these newly discovered evolutionary significant units and their corresponding habitats.

Hybridisation and chromosomal rearrangements speed up adaptation and speciation

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Speciation rates vary massively across the tree of life. Some lineages diversify rapidly into many species, whereas their close relatives speciate at a much slower pace in the same environment. The factors influencing such lineage-specific speciation rates are poorly understood. Hybridisation and chromosomal rearrangements are two of the factors that may contribute. Each of these factors can both increase or reduce the likelihood of speciation.

Together with a network of collaborators in the Americas and Europe, we study the role of hybridisation and chromosomal rearrangements in speciation in ithomiini and *Heliconius* butterflies. These neotropical butterflies are well-known for their Müllerian mimicry rings and high species richness. The large variation in speciation rates across different lineages, particularly within the ithomiini tribe make them an ideal system to study factors affecting speciation rates. Ithomiini and one lineage of *Heliconius* butterflies also show high rates of chromosomal evolution with karyotypes ranging from five to 120 chromosomes (Brown et al. 2004). By combining population genomics approaches and chromosome-level reference genomes we elucidate the roles of hybridisation and large-scale chromosomal rearrangements in speciation.

Genomic data reveals strong differentiation of populations in co-occurring sister *Spicauda* species (Hesperiidae)

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Evolutionary biology and community ecology suggest that, to co-occur, closely related species must have reached stabilizing niche differences to reduce competition, and complete reproductive isolation to maintain such species differentiated over evolutionary time. To investigate the aforementioned prediction, we used a multidisciplinary framework to infer population history and recent genetic differentiation dynamics in four syntopic, sister species in the genus *Spicauda* (Hesperiidae: Lepidoptera) in two neotropical localities: Panamanian lowland and Peruvian Andes premontane forests. These species are widespread across the Neotropics and co-occur in several sites, they present high morphological similarity (brown wings and hindwing tails), and presumably feed on similar larval hostplants, and share avian predators while flying usually in similar environments at similar times. We use whole-genome sequencing data to, first, infer their time-calibrated phylogenetic relationships using secondary calibration points. Second, we estimated population differentiation within and between localities as a proxy for the strength of reproductive isolation. We found no signs of introgression or hybridization, nor discordance in mitogenomic and nuclear evolutionary histories, among co-occurring *Spicauda* species, despite their recent divergences (~8 million years ago) and syntopic occurrence. Future studies will uncover potential intrinsic barriers to gene flow, both prezygotic (e.g., pheromone differentiation) or postzygotic (e.g., chromosomal rearrangements), that are leading to such degrees of differentiation among *Spicauda* species. Our study brings a holistic view on the macro- and micro-evolutionary drivers of the co-occurrence of sympatric and syntopic species in natural populations.

Species delimitation under allopatry – Patterns of intraspecific genomic divergences within and across the continents in Lepidoptera

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Delimitation of allopatric populations into species is difficult and largely arbitrary. Particularly variation in morphological and genetic characters in space and time creates significant subjectivity for delimitation of taxa, thus leading to taxonomic instability. Many species found both in alpine and arctic habitats fall into this category because their ranges are fragmented owing to the discontinuous distribution of their habitats. The same concerns the many Holarctic species, which almost regularly show at least some differentiation between the continents. In this study, we analyze high-throughput target enrichment data for ten groups of arctic-alpine and Holarctic lepidopteran species sampled across the Holarctic realm – *Elophos vittaria*, *Eudonia sudetica*, *Syngrapha hochenwarthi*, *Xestia speciosa*, *Agriades glandon*, *Arctia caja*, *Macaria brunneata*, *Carsia sororiata*, *Coenonympha tullia*, *Xestia lorezi*. We first assessed whether DNA barcode divergences reflect the genetic differences in the nuclear genome. Secondly, we tried to pinpoint whether the difference between population and species-level differences can be identified using genomic data, using a suite of population genetic and species delimitation analyses. Our result suggested mitonuclear discordance and possible species-level differentiation of the Nearctic population and Palearctic alpine and arctic populations in some cases, but not in all. Finally, we also discuss the results in the light of past climatic oscillations that have shaped the diversity of arctic-alpine and Holarctic Lepidoptera.

POSTER PRESENTATIONS

Butterfly communities

The importance of moth pollination in tropical ecosystems

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The importance of moths in tropical pollination still needs to be better understood. We reviewed the available literature from tropical ecosystems to assess moths' importance in the pollination of plant species and communities. Moths have a well-adapted olfactory system and vision. Although several moth taxa have reduced mouthparts in the adult stage and do not feed, the nectarivorous moths use vision and smell not only to find a partner for copulation but also to locate a nectar source in the dark environment. Although plants produce nectar to reward pollinators for their service, from the pollinator's perspective, flower pollination is usually just a by-product of feeding on resources. Functional pollinator groups (such as flies, moths, bats, and bees) significantly differ and visit variable flowers. Plants convergently evolved complex sets of adapted floral traits – called pollination syndromes – to attract particular pollinators. Numerous plant taxa have specialised sphingophilous and phalaenophilous (hawkmoth-attracting and moth-attracting pollination syndrome, respectively) flowers. Our review revealed that moths are dominant nocturnal visitors of moth-attracting flowers in the tropics. Plant species with moth-adapted flowers are, however, uncommon in plant communities. In highly specialised plants, moths often serve as the only pollinators. However, plants pollinated primarily by moths are relatively rare in plant communities. Nocturnal pollination is still strongly understudied, despite moths being crucial pollinators of numerous plants. More comprehensive pollination studies are needed for a better understanding of the role of moths as pollinators in tropical communities.

Lepidopteran fauna of natural mountain spruce forest in the Trojmezná (Šumava: Czechia)

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Natural mountain spruce forests belong to the most endangered biotopes in central Europe. We bring contribution to the knowledge of the lepidopteran fauna before the forest stand disturbance and decline of the old-growth spruces in that area. Data used rely on captures in the Malaise trap during 2003–2004 and collections into portable light traps or light sheet in 2006–2007 in the Trojmezná massif, at elevations 1200–1300 a.s.l. It was found 290 species of Lepidoptera, most in the family Geometridae (80), Noctuidae (63), Tortricidae (26) and Pyralidae (20). Highest density reached *Entephria caesiata*, *Eulithis populata*, *Hydriomena furcata*, *Dysstroma truncata* and *Operophtera brumata* (Geometridae); *Pseudohermenias abietana*, *Epinotia tedella* and *Ancylis myrtillana* (Tortricidae); *Elachista bifasciella* (Elachistidae). A close relation to the mountain spruce forest from the species found could be considered for *Hepialus fusconebulosus*, *Denisia nubilosella*, *Elachista bifasciella*, *Chionodes luctuella*, *Lozotaenia forsterana*, *Cydia indivisa*, *Eudonia petrophila*, *Udea decrepitalis*, *Cosmotriche lobulina*, *Elophos vittarius*, *Xanthorhoe incursata*, *Entephria caesiata*, *Thera vetustata* and *Xestia speciosa*. From rare and unusual species were caught *Incurvaria vetulella*, *Zelleria hepariella*, *Buvatima stroemella*, *Agonopterix angelicella*, *Agonopterix petasitis*, *Caryocolum cassella*, *Martania taeniata*, *Eupithecia actaeata*, *Mythimna anderegii* and *Basistriga flammata*. Although spruce forests are generally considered biotopes with low diversity, natural mountain spruce forests contain relatively rich and interesting lepidopteran fauna.

Butterfly diversity under climate change

Past, present, and future of the NW Spanish montane endemism *Erebia palarica* Chapman, 1905

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Alpine and subalpine biodiversity is particularly vulnerable to the rapid oscillations in environmental conditions derived from climate change. Recent modelling showed that only three of the 19 species of *Erebia* (Nymphalidae, Satyrinae) currently present in the Iberian Peninsula would remain there in the worst-case scenario for 2070. One of them is *Erebia palarica*, endemic to the mountain ranges of Northwest Spain. We performed an integrative analysis combining genetic and morphometric approaches, using samples from the complete distribution range to determine the evolutionary history and current population structure of *E. palarica*.

We sequenced a 786bp fragment of the mitochondrial Cytochrome Oxidase I (COI) gene in 305 individuals and identified 24 haplotypes. The 95% statistical parsimony network revealed a double star-like pattern connected by a single mutational step. This result points towards a demographic expansion from two ancestral populations. This finding is congruent with the larger putative distribution area revealed by climatic modelling during the Last Glacial Maximum.

The current population structure was inferred after genotyping 383 individuals with seven microsatellite markers and a morphometric analysis of wings. Firstly, we detected a single cluster of 194 individuals (central-eastern part of the Cantabrian range) mostly affected by isolation by distance. Secondly, we found different levels of differentiation in the western localities. Actually, the westernmost site (Queixa, previously described as a subspecies), was recovered as a clearly separate unit by genetic and morphometric analyses. Lastly, the different effects of sex and locality on the variability of wing size, shape and pattern will be discussed.

Butterfly monitoring, trends and indicators

Building a Global Butterfly Index

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There is a taxonomic bias in conservation research towards vertebrates, resulting in limited data on status or trends for many insect groups. Where data exist, e.g. Europe and the USA, some large butterfly population declines have been recorded. Without sufficient monitoring elsewhere, we may be unable to recognise population declines and implement the required conservation actions.

The Global Butterfly Index is a collaboration project aiming to gather time-series population data from butterfly monitoring schemes around the world to create an indicator of global butterfly population trends. We hope that the results will:

- Create a global indicator to detect population changes which is less biased towards Europe and North America,
- Provide a mechanism to understand drivers of change across all biogeographic regions, and
- Establish an invertebrate group firmly in the global biodiversity indicator and policy space (including the Living Planet Index), so that the findings will have impact.

The Global Butterfly Index project is in its early stages, and we are looking for information about and data from butterfly monitoring schemes. This map summarises those we are aware of so far.

Could you add any information to this map?

How weather and body size affect survival, senescence and detectability in a natural butterfly population

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To deepen our understanding on the dynamics of butterfly populations and to improve our monitoring methods, it is inevitable to reveal how environmental variables and body size are related to survival, senescence, and detectability of butterflies.

We aimed to reveal these relationships with a thorough mark-recapture study in a population of the univoltine Clouded Apollo (*Parnassius mnemosyne*) butterfly in Hungary. Sampling was conducted from 2014 to 2019 during the whole flight period each year. We measured body mass, thorax width, forewing length, and proboscis length *in vivo* on individually marked butterflies. Temperature and relative humidity were measured by loggers installed for the whole year in the field. We analyzed data using Cormack-Jolly-Seber models including weather and body size variables as covariates.

In all years, butterfly survival declined with age or with the progress of the flight period in both sexes. Generally, at lower temperature, higher relative humidity, and with larger body size, individuals had higher survival probabilities, but these relationships were significant only in a few years. Higher temperature also accelerated aging in some years.

Recapture probability increased with temperature, number of observers, body size and age of butterflies, and it declined with relative humidity. However, these relationships were also detectable in some years only. We found relatively small differences between the sexes, but a high annual variation in the ranking of models. Our results provide a deeper insight into the demography of butterfly populations and may enable us to reveal potential pathways of adaptation to environmental changes.

The potential of timed area counts for monitoring butterfly populations across Europe

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Effective biodiversity monitoring is crucial for tackling the biodiversity crisis – more representative trend assessments, identifying major causes of change and to evaluate conservation approaches. All monitoring methods have trade-offs between scientific value versus potential for long-term and large spatial scales applications. Opportunistic recording has a simple approach that reduces barriers to participation, engages large numbers of participants and is primarily used to measure range changes. Standardized Butterfly Monitoring Schemes require more effort but generate rich abundance datasets for biodiversity indicators and many research applications. Despite rapid growth and high value of these two main approaches, assessment of butterflies in Europe has considerable spatial, temporal and taxonomic biases. Through European Butterfly Monitoring Schemes, with support from EU-funded projects (ABLE, SPRING), we have developed a Smartphone application (ButterflyCount) to enable more widespread capture of standardized butterfly abundance data. We review the uptake of the application, focusing on case studies that use 15-minute timed area counts to improve coverage in under-represented habitats (e.g. urban and farmed environments, remote areas) and for under-represented species (e.g. rare and habitat-specialist species). We assess how combination of monitoring approaches can further improve assessment of butterfly populations across the EU, in support of research and conservation.

What functional traits of moths determine anthropogenic habitats?

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Rapid economic development, climate change, agriculture intensification and industrialisation have a crucial impact on species diversity. An important tool for studying biodiversity in the context of environmental change are the functional traits. The study was conducted near the town Horní Bříza, Pilsen Region, Czech Republic, an important centre of mining of kaolin, a clay mineral for porcelain, cement and ceramics production. We sampled the moths using portable light traps. Four sites were situated in the mining area where a kaolin is still extracted, four other sites were outside of the active part of the quarry. Within these sites, four sites were characterized by spontaneous vegetation succession and other four sites were reclaimed with pine trees. We analysed the moth catches using such functional traits as wing span, overwintering stage, flight period length, host plant form, host plant part, humidity preferences and voltinism. We found 178/983 moth species/individuals. Testing categorical predictors showed the significant differences in moth assemblages between the mined and unmined sites. Only the trait *host plant form* significantly differed between the assemblages, so that moths developing on trees prevailed outside of the active part of quarry, and species developing on forbs and grasses prevailed on early successional habitats within the active part of the quarry. The results document that disturbance created by the mining supports moths associated with early successional vegetation.

Conservation of butterflies and their habitats

Chasing butterflies across the sands: towards an understanding of population density and habitat use by the Sand Mountain blue

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Even in areas with low human population density, conservation often entails a compromise between various demands on natural resources and the ecological requirements of focal species. Sand Mountain is a dune system of nearly 5,000 acres located in central Nevada, USA. The mountain is a popular recreation area for off highway vehicles as well as being home to the only known population of *Euphilotes pallescens arenamontana* (Sand Mountain blue). In 2006, the U.S. Fish and Wildlife agency petitioned for the Sand Mountain blue to be listed as endangered, but the petition was found to not be warranted, and monitoring of the butterfly was not continued. The main objective of my work is to assess the current status of the Sand Mountain blue population and to improve our understanding of habitat use in this unusual landscape. Although it is preliminary data, the previous season of fieldwork suggests that the Sand Mountain blue population is at a lower density compared to 2006 when the last surveys took place. Population estimates are based on a 2.5 mile long transect with 14 sites where two 5-minute wandering surveys occur per site and adults are counted. I will also describe ongoing efforts to understand pollination of the host plant, *Eriogonum nummulari*, and to census caterpillars and model caterpillar habitat use as (potentially) a subset of habitat used by adults. Results from this work will hopefully inform management of the area and the sensitive butterfly species.

Five Years of Reintroduction of Chapman's Blue in Prague

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Reintroduction programs to save endangered species of butterflies provoke conflicting reactions in society. On the one hand, they are taken as one of the established means of modern nature protection, but on the other hand, they are rejected due to frequent failures, interventions in endangered communities or as a source of noise when mapping the occurrence of autochthonous populations (especially "black" reintroductions). For these reasons, it is therefore appropriate, in cases where it is only slightly possible, to create a new environment for the reintroduced populations without burdensome conflicts associated with the previous history of the sites. Such projects include the reintroduction of the Chapman's blue (*Polyommatus thersites*) in Prague. Populations of this species have rapidly decreased due to habit change and loss over the last decades. Nowadays it is one of the most vanishing species of butterflies in the Czech Republic. The same story was with its last population in the capital city of Prague, where the last specimen was observed in 2004 after intensive monitoring. In 2018 the reintroduction experiment was started on newly created meadows from formerly arable lands near Prokop Valley protected area. Since few individuals (< 100) during the beginning, now the viable population were established with estimated counts of ca. 3000 individuals of spring generation in 2022 and with well-documented arbitrary dispersions to the close surroundings. The example of Chapman's blue reintroduction in Prague literally in a former rape field demonstrates the high potential of places with minimal biological value before their modification.

Conservation of *Parnassius apollo* in Poland, Czech Republic and Austria (LIFE APOLLO2020)

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Parnassius apollo has become a flagship species for nature conservation. Despite conservation and reintroduction attempts, the species is considered extinct in the Czech Republic, although one reintroduced population has been successfully established in the Štramberk quarry. In Poland, a single population in the Pieniny Mountains has been successfully stabilized but attempts to reintroduce the species in the Sudety Mts. were unsuccessful in the past.

The project is the first attempt at a coordinated conservation project for *P. apollo*, involving practitioners from three Central European countries. The main goals are to (1) restore the populations in two functional areas: the Sudetes and White Carpathians, and strengthen the populations in the Austrian Alps in the Continental bioregion, (2) initiate the process of creating metapopulations in these areas, by creating around 40 “stepping-stone sites” to secure species migration, (3) create three additional breeding farms in all three functional areas to anchor the population in natural conditions, as well and (4) raise awareness and social commitment to the conservation and maintenance of species’ habitats by establishing 50 “Apollo Gardens” with involvement groups of locals and volunteers and organizing youth education workshops. The project has also a strong scientific aspect, by integrating the genetic analyses of past and current populations in conservation planning. Finally, the project will establish a coherent species conservation approach at the Continental bioregion level with groups of experts from the relevant countries and disseminate the results in a form of a Breeding and Conservation Manual.

Eco-evo-devo of lepidopteran color patterns

Exploring genetic variability and its potential influence on wing color patterning in *Bicyclus anynana* butterflies: A focus on allelic variance

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Genetic diversity and allelic variation are crucial for organism adaptability and survival in different environments. Advances in genetic engineering tools have allowed deeper understanding of metabolic, physiological, chemical, and phenotypic processes. Butterflies have high genomic variability, which has been vital in determining distinct wing patterns. The *yellow* gene plays an essential role in pigmentation determination, is highly conserved in several insect species, and widely studied in wing color patterning and evolution. Allelic variance in exon 3 of *yellow* gene and its relation to wing color determination in the *Bicyclus anynana* butterfly were evaluated. Surprisingly, in the wild type population, we found allelic variation in exon 3, with four different homozygous alleles. The most common allele was found in 42.6% of the population, followed by 37%, 15% and 5% respectively. In comparison, *yellow* mutant lines generated through CRISPR/Cas9, had four homozygous allelic variants with 62%, 34%, 2% and 2% prevalence, respectively, after knocking out exon 3. The allelic variants between wild type and *yellow* mutants are different, suggesting that this allelic variance in exon 3 of the *yellow* gene may have an influence on wing color patterning, even in wild type populations. We tested this hypothesis by analyzing wing reflectance of males and females spanning exon 3 variation in both WT and *yellow* mutant populations, with a focus on background coloration on dorsal and ventral wings. The results of this study provide new insights into the genetic basis of wing color determination in butterflies.

Speak for the trees: Phylogenetic mapping of eyespots across multiple superfamilies of Lepidoptera

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Eyespots are evolutionary innovations in Lepidoptera, however, determining their origin has proven challenging. To date most research has revolved around family Nymphalidae, where eyespots are demonstrated to have a single origin. Using modern phylogenetic techniques and the most extensive multi-superfamily Lepidopteran tree to date, this study aims to identify the evolutionary origins of two main types of eyespots in Lepidoptera. Here eyespots are defined as two or more concentric rings of colour, sub-divided into discal (occurring on discal wing regions) and vein (occurring between wing veins) eyespots. The occurrence of eyespots in this study was recorded across 715 extant Lepidoptera from 29 superfamilies, 90 families and 639 Genera. Number and phylogenetic placement of independent eyespot origins were inferred using ancestral state reconstruction and marginal likelihood approximation. We observed two distinct families of Lepidoptera (Nymphalidae, Saturniidae) where both discal and vein eyespots evolved and expanded, in addition to 28 potentially independent origins across other Lepidoptera. In Saturniidae we observe that evolution of discal eyespots preceded vein eyespots, and *vice versa* in Nymphalidae. Across other lineages, discal and vein eyespots were present independently. Many species presented dual eyespot phenotypes (discal and vein). Discal eyespots have 26 independent occurrences and vein eyespots have 20. Our results demonstrate that eyespots have evolved independently in Lepidoptera and are not restricted to select lineages. We also show that discal eyespots occur more commonly than vein eyespots. Interesting avenues for further research include investigating whether the observed independent eyespot origins rely on shared or unique genetic mechanisms.

The genetic basis of wing spots in *Pieris canidia* butterflies

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Spots in pierid butterflies and eyespots in nymphalid butterflies are likely non-homologous wing colour pattern elements, yet they share a few features in common. Both develop black scales that depend on the function of the gene *spalt*, and both might have central signalling cells. This suggests that both pattern elements may be sharing common genetic circuitry. Hundreds of genes have already been associated with the development of nymphalid butterfly eyespot patterns, but the genetic basis of the simpler spot patterns on the wings of pierid butterflies has not been investigated. To facilitate studies of pierid wing patterns, we report a high-quality draft genome assembly for *Pieris canidia*, the Indian cabbage white. We then conducted transcriptomic analyses of pupal wing tissues sampled from the spot and non-spot regions of *P. canidia* at 3–6 h post-pupation. A total of 1352 genes were differentially regulated between wing tissues with and without the black spot, including *spalt*, *Krüppel-like factor 10*, genes from the Toll, Notch, TGF- β , and FGFR signalling pathways, and several genes involved in the melanin biosynthetic pathway. We identified 14 genes that are up-regulated in both pierid spots and nymphalid eyespots and propose that spots and eyespots share regulatory modules despite their likely independent origins

Genome structure and evolution

A role of sexually antagonistic selection in sex chromosome evolution of a common blue *Polyommatus icarus* (Lycaenidae)

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The classic model of sex chromosome evolution assumes that sex chromosomes evolved from a pair of autosomes harbouring sex determining locus. Sexually antagonistic (SA) selection, i.e. selection for an allele beneficial to one sex but deleterious to the other, should favour linkage between SA allele and sex determining locus and further suppression of recombination and subsequent differentiation of sex chromosomes. In Lepidoptera, W chromosome evolved most probably through adoptions of a B chromosome and thus the classic model of sex chromosome differentiation can be applied only to neo-sex chromosomes resulting from sex chromosome-autosome fusions and translocations. The neo-sex chromosomes are quite common and occurred independently in various lepidopteran taxa.

We performed cytogenetic and bioinformatic analyses of *Polyommatus icarus* sex chromosomes and detected two karyotypic races which differed in their neo-sex chromosomes. The sex chromosome turnover and early stage of differentiation of its neo-sex chromosomes make *P. icarus* a suitable model for testing a role of SA selection in sex chromosome-autosome fusions. Due to achiasmatic meiosis of lepidopteran females, it is not possible to directly detect SA selection from elevated relative sequence divergence between sex chromosome pseudoautosomal regions. However, it is possible to test for SA selection indirectly by expression profiling. We performed RNAseq from early instar larvae and imaginal tissues of both sexes and used it to identify SA genes in *P. icarus*. We studied a role of SA selection in neo-sex chromosome evolution by testing whether the fused autosomes are enriched in genes with SA expression.

Development of molecular markers for the W chromosome in the blue butterfly *Polyommatus icarus*

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Unlike most other lepidopteran groups, blue butterflies of the family Lycaenidae show great variability in the number of chromosomes, which arises due to repeated fragmentation of their chromosomes. Another chromosome rearrangement observed in the blue butterflies was sex chromosome-autosome fusions resulting in the so-called neo-sex chromosomes. Such a derived system was found in the common blue butterfly, *Polyommatus icarus*. Moreover, two karyotype races differing in the number of sex chromosome-autosome fusions were found in Czech *P. icarus* females, which makes the species a suitable model system to study drivers of neo-sex chromosome evolution.

The fixation of neo-sex chromosomes in butterflies may be facilitated by sexually antagonistic selection, i.e. selection for alleles beneficial to only one sex and potentially harmful to the other. To test this hypothesis, molecular markers for easy sex determination in the early stages of development are needed.

Through comparative k-mer-based bioinformatics analysis of resequencing genomic data of both sexes, we identified sequences derived from the W sex chromosome and designed primers for molecular sexing of *P. icarus*. A functional pair of primers was found that allows for reliable sex determination of *P. icarus* individuals simply by means of PCR. The sexing was used to determine the sex of freshly hatched *P. icarus* caterpillars used for RNA-seq and subsequent differential expression analysis. The presented pipeline could be also used in other species.

Museomics to understand the phylogenetic relationships and wing-pattern evolution of burnet moths (Zygaena)

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Burnet moths (Zygaenidae: Zygaena) are amongst the most striking and recognisable Palaearctic Lepidoptera. They are diurnal with bright aposematic colours and show extensive intraspecific and interspecific variation in wing pattern. Currently, 108 species are known but nearly 730 subspecies have been described to cover the entire phenotypic variation in the entirety of the geography. Despite being subject to much historical collecting interest and the current detailed knowledge of many populations, there has been relatively little work on the phylogenetic relationships in this group, especially based on molecular data. To clarify the systematics, our project sets to produce a molecular phylogeny including all species and several hundred subspecies using medium coverage high-throughput sequencing. Besides fresh samples, this study has a strong component of historical collection-based genomics, or Museomics, setting to decipher the relationships of little known, unique or long-gone taxa. Furthermore, we set to study the relationships amongst the different wing-pattern ground plans and the evolution of wing pattern across burnet moths both using traditional morphometrics and machine learning approaches.

Order-wide dynamics of gene evolution across lepidopteran genomes

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Lepidoptera represent one of the most diverse animal orders with over 160,000 described species. The diversity in form and behaviour within this clade can be attributed to adaptations at the genetic level. Studies of gene content and genome evolution in Lepidoptera have been limited to certain well-studied species, owing to the lack of high-quality genome data for most families. Recent construction of a large number of chromosome-level assemblies provides the opportunity to analyse the role of gene and genome evolution in the diversification of this Order of insects. Here, we provide a genome-wide analysis of protein coding gene evolution across Lepidoptera. We assess order-wide rates of evolution, patterns of gene gain, duplication and loss, and conservation of gene microsynteny across 216 arthropod species, 182 of which are Lepidoptera. Comparing rates of evolution between families reveals variable patterns of genetic diversity, with the families Pieridae, Lycaenidae, and Geometridae displaying the highest overall genome-wide evolutionary rates. Assessing gene turnover reveals dramatic bursts of gene gain in the two nodes subsequent to the early diverging families Micropterigidae and Tineidae (i.e. Ditrysia minus Tineidae and Apoditrysia), with 1,623 and 1,444 gene families gained, respectively. Finally, we analysed the conservation of local gene synteny across Lepidoptera. Similar to genome macrosynteny, we find a high rate of conserved microsynteny across the Order, as well as family specific gene clusters, for example in Tortricidae (671 gene clusters), Lycaenidae (649 clusters), and Geometridae (418 clusters). These may provide insights into family specific gene reshuffling, clustering, and regulation.

Genomics of local adaptation in the widespread butterfly *Melanitis leda*

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Species that are widely distributed have characteristics that enable them to colonize numerous areas where they are subject to selection pressures brought on by a variety of habitats. The aim of the study is to determine how the widespread Satyrine butterfly, *Melanitis leda*, has colonized its range and adapted to varying climates and habitats. This species is especially interesting because it shows seasonal polyphenism while occurring in various climate zones, and dry season forms are highly polymorphic. To be so successful, the species might possess phenotypic plasticity that functions well in many climates and habitats, or consist of locally adapted populations. The first step in my research is to generate a reference genome. Secondly, I aim to reconstruct the phylogeography and demography of the species. Finally, I aim to identify genes that are associated with adaptation to habitat, in particular grassland vs forest, and to determine whether such adaptations use the same alleles across regions, or rather use independent mutations. The *de novo* genome assembly is under way, and I am gathering samples from all over its range (spanning in countries of South Africa, South and South East Asia, Oceania), and habitats including forest and grassland habitats for whole genome resequencing.

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Migration in butterflies

Species Distribution Modelling and Remote Sensing Vegetation Indices to study migratory butterfly seasonal movements and population outbreaks

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Dispersal and migratory movements of phytophagous insects such as butterflies are strongly tight to vegetation phenology. The succeeding broods of seasonally migrating species connect distant regions that differ in the timing of plant growth. Also, the overall plant production and the extent of the growing season determine the breeding capacity of the insects, influencing their demographic trends. Photosynthetic activity proxies based on remote sensing observations (e.g. NDVI, EVI) open new opportunities to study insect migration. Here, we present two applications of NDVI to study the migration of *Vanessa cardui*, an obligate migratory butterfly across its Afro-Palearctic range. We develop a spatio-temporal Species Distribution Model (SDM) using time-series of climatic and vegetation variables, including (1) EVI and (2) monthly EVI difference. EVI proved to be a relevant variable explaining shifting breeding suitability, with higher importance in the Afrotropics. In the Palearctic, suitability was mainly explained by variables related to temperature. At a temporal scale, we assess the role of NDVI anomalies in modulating demographic trends of *V. cardui* through time-series analysis of monthly NDVI values from 2001 to 2021. We observe that four butterfly outbreaks in Europe were immediately preceded by anomalous vegetation growth in suitable breeding regions of Africa and/or the Middle East, suggesting a strong association between both events. Taken together, we highlight the potential of remote-sensing vegetation indices to study seasonal distributions of migrating phytophagous insects and how NDVI anomalies can be used to predict potential population outbreaks.

How does landscape structure affect butterfly flight capability?

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Land use is one of the key drivers in the current biodiversity change with agricultural intensification impacting natural habitats worldwide. Dispersal ability influences species ability to survive and adapt to changes in their surrounding landscape and studies assessing among species variation in dispersal suggest that species with better dispersal abilities are more likely to survive these changes resulting from intensified land use. Less is known about land use impacts on within species dispersal ability.

Flight metabolic rate has been shown to be a good proxy for dispersal ability in butterflies. In this study we will measure variation in flight metabolic rate from wild-caught individuals of four butterfly species of varying body sizes and population trends, latter which is assessed based on long-term monitoring data. Individuals will be caught from two different types of habitats; habitats surrounded by agricultural fields and more natural or semi-natural meadow habitats in the Åland archipelago, South-West Finland during the summer of 2023. I will present some preliminary results on one of the selected species, *Pieris napi*, which is a bivoltine species in Finland and has an early summer first generation.

The results of this study will give us more information on the effects of landscape structure on performance traits of butterflies. They will also provide insight on whether morphological traits correlate with the flight metabolic rate and how much within and among species variation there is in dispersal.

A little-known dimension of genital diversity in female Lepidoptera: the bursa muscularis

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The bursa muscularis is the set of muscles associated with the bursa copulatrix of the female reproductive system in Lepidoptera. Its delicate structure, difficult to access, has been a neglected issue in lepidopterological research. The combination of modern techniques of scanning electron microscopy and confocal microscopy with image processing as 3D reconstruction and stacking contributes to exploring and understanding its structure and function. The bursa muscularis is sometimes trivialized, as associated with the signum and its mechanical properties in the rupture of the spermatophore. However, the bursa muscularis may or may not be associated with the presence of signum and in some cases it may be completely absent. Certainly, it is associated with specific movements of discrete parts of the bursa copulatrix but with a strong and diverse functional component. Its structure is complex, shows relevant variation and cannot be always directly inferred from the skeletal morphology of the bursa copulatrix. Bursal muscles may in virtually the entire series of reproductive events from mating and copulation to handling the spermatozoa and other substances after copulation. Bursal muscles are attached to significant areas of the bursal integument and must be ready to respond to a variety of stimuli including stretch. Here we present a summary of the state of the art and future lines of research in the study of the bursa muscularis in an evolutionary perspective.

Evolution of wing pattern in the *Zygaena manlia* group using machine learning and phylogenomics

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The genus *Zygaena* (Fabricius 1775), a diverse group of day-flying moths belonging to the family Zygaenidae, has remarkable diversity in wing patterns and geographic ranges. However, the evolutionary history of this genus has remained largely unresolved, despite its ecological and evolutionary significance. The most species-rich group of *Zygaena* is the *Z. manlia* group, which is restricted to an area ranging from Anatolia to the Hindukush and currently comprises 21 recognized species. This group has undergone very recent radiation, but the phylogeny of this group is not fully resolved.

To address this knowledge gap my project aims to understand the evolution and study the morphological variation between species of the *Z. manlia* group. Whole genome sequences will be used to resolve the phylogeny, while computer vision and machine learning techniques will be employed to build a species classifier model. This model will be trained to identify images of museum specimens using the entire specimen, wing spot pattern alone, and both with and without geographic location information to improve identification accuracy.

Morphological comparisons of the extracted wing spot features such as color, shape, and patterns can also be made. Geographic data will be correlated with the extracted features to identify any potential patterns. This study will provide insight into the evolutionary history of the *manlia* group and contribute to the understanding of the origins, diversification, and adaptation of species.

Winter moths on co-occurring oaks and hornbeams are isolated by adaptation to budburst phenology

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Phenological matching plays a crucial role in the performance of herbivorous insects. Neighboring tree species in a forest show differences in budburst timing that may lead to demographic isolation of populations feeding on them. The larvae of winter moth (*Operophtera brumata*; Geometridae) hatch in spring and feed on developing leaves of a range of deciduous trees and shrubs. In the temperate zone, oak (*Quercus* sp.) is one of its main hosts. We studied the association between the budburst phenology of co-occurring tree species with early (hornbeams) and late (oaks) budburst, and the abundance and hatching date of winter moths. The winter moth populations on oaks in the Puszcza Zielonka forest in Poland had crashed in 2018 and they were recovering in neighborhoods with hornbeams. Winter moths remained rare in stands where hornbeams were absent. The vast majority of winter moths showed early egg-hatching when both hornbeam and oak were present, which matches the early budburst of hornbeam. Eggs typically hatched late where hornbeam was absent, matching the later budburst of oaks. Our results suggest that winter moth populations on hornbeam and oak are isolated from each other due to their adaptations to the different budburst phenology despite spatial proximity, so that recolonization of oaks from hornbeam-adapted populations is slow

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Phenotypic plasticity in butterflies

How do *Panacea prola* and other Amazonian butterflies adapt to natural seasonal cycles and how will this help them to cope with anthropogenic climate change?

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The southwest of the Amazon witnesses a large change in climatic conditions throughout the year, with a pronounced dry season. Despite their ecological importance and the immense local biodiversity, the responses of insect herbivores (including *Panacea prola* and other butterflies) to seasonality here are relatively unknown.

This project aims to determine the adaptations and responses of butterfly communities to seasonality and to identify the key environmental drivers. The two main responses I will be studying are migration (particularly in the species *Panacea prola*), and reproductive diapause in the wider butterfly community.

To analyse patterns of migration in *Panacea prola*, I will analyse iNaturalist and other observation data and integrate these with stable isotope analysis. In addition, I will quantify migratory behaviour using tethering experiments in the field. Then, to identify the drivers of this behaviour, I will collect biotic and abiotic data during the change from wet to dry season.

At the community level, I will quantify seasonal reproductive diapause in the community through dissections of wild-caught females. I will also analyse them in a phylogenetic comparative framework and integrate ecological and climate data to identify key drivers of this adaptation.

Climate change is likely to exacerbate the selection pressures faced by the Amazonian butterfly community, and therefore understanding their plastic and evolutionary responses is incredibly important for their future conservation. My work will contribute to conservation management decisions in the southwest Amazon moving forward.

Effect of Mating on Male Choosiness in *Bicyclus anynana*

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Males mate multiply in many species, including butterflies, but little is known about whether the act of mating influences future male choosiness. We might expect mating to influence future preference, as previous studies have shown that pre-mating social experiences affect future male mate choice. To determine if initial male mate choice affects subsequent male mating decisions, we conducted behavioral assays using the subtropical nymphalid butterfly *Bicyclus anynana*. We tested three alternative hypotheses: 1) initial mating is a positive experience that strengthens male preference for the previously encountered phenotype; 2) males prefer mating with a different phenotype in their second mating, exhibiting bet-hedging behavior; and 3) there is no effect of past mating experience on future mate choice. We first mated *B. anynana* males with a female with 0 or 2 dorsal hindwing eyespots (DHS). We then allowed males to choose between females with 0 or 2 DHS to determine if the initial mate's wing pattern influences male preference for that female's wing pattern in subsequent mating encounters. We utilized paint to alter wing ornamentation by either painting over UV DHS or painting black spots below existing UV DHS. We used naïve male preference as a control. We found that males from all three treatments mated randomly when choosing between females with 0 and 2 DHS. Our findings suggest no effect of past mating experience on future mate choice in *B. anynana*, implying an essential distinction between pre-mating exposure and mating exposure in the male *B. anynana* brain.

Novel food odor learning across generations and inheritance of learned odor preference via hemolymph transfusions in *Bicyclus anynana* butterfly larvae

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Several herbivorous insects have specified preferences for their host plants and it is uncertain how these preferences have evolved. Earlier on, we found that *Bicyclus anynana* larvae, when fed on novel odor-coated leaves, can learn to prefer that odor and also transmit that learned preference to their offspring. Yet, it is unclear if that learned preference can be stabilized/maintained in the absence of the odor. Another unknown in this study is the epigenetic mechanism by which this learned preference is being inherited. In an aim to address the former question, we fed larvae with leaves coated with a novel banana odor for five generations. After that, the odor treatment larvae were separated into two subgroups, in which one had the odor removed, while the other group was continued to be odor-fed. We found that five generations of continuous odor exposure did not lead to the stabilization/genetic assimilation of that learned odor preference. Next, we performed hemolymph transfusions from odor-fed to naïve larvae. The injected larvae did not show immediate changes in their innate odor preference. However, we found that their offspring preferred odor more in comparison to the offspring from parents that were injected with hemolymph from control-fed larvae. This finding suggests that hemolymph carries factors that have an important role in generational transmission of learned odor preference. This study throws light on the fact that there might be mechanisms that mediate epigenetics, other than the commonly studied ones such as DNA methylation and small RNAs inheritance.

Population, landscape, and conservation genetics

Census and contemporary effective population size of a population of *Erebia palarica*, Chapman, 1905

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Butterflies are highly sensitive to oscillations in environmental conditions, particularly those with restricted habitats or narrow ecological requirements. Such is the case of the endemic *Erebia palarica*, limited to the mountain ranges of Northwest Spain and classified as “data deficient” by IUCN. In the present work, we studied *E. palarica* from Serra do Courel (Lugo province, Galicia region), a relatively isolated area, to shed some light on the conservation status of the species.

We firstly conducted a mark-release-recapture (MRR) study during summer of 2021, gathering data regarding mobility, behaviour and phenotypic variation for 707 butterflies. We surveyed two localities of Serra do Courel separated by 3.26 km (Alto do Couto and A Cabeza Grande) and obtained census (N_c) estimates of 565 and 303 individuals, respectively. The extrapolation of our results to the complete distribution area confirms the status of *E. palarica* as of “least concern” following IUCN standards.

Secondly, we produced a fine-scale distribution map in Serra do Courel. We confirmed the presence of the species in various localities cited in the literature, and detected the butterfly in new nearby areas with favourable habitats. Additionally, our field observations suggest low dispersal rates in *E. palarica* as well as territorial behaviour in males.

Lastly, we will next look at genetic variation as revealed by seven microsatellites for a time series of 137 samples obtained between 1998 and 2021 in Alto do Couto. These data will allow the calculation of contemporary effective population size (N_e) using both single-sample and temporal approaches.

Systematics and biogeography of Lepidoptera

Diversification of phyto-predaceous lycaenid butterflies (Polyommatainae, Lycaenidae) during the Miocene aridification of the African continent

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Over the past 20 Myr, Africa became increasingly arid, leading to both radiation and extinction of species. The mechanisms behind this are poorly understood. Phyto-predation, a rare life history where caterpillars feed first on plants, then on ant brood, may have favored the explosive diversification of the afrotropical genus *Lepidochrysops*. It is hypothesized that ant nests provide shelter from fire and drought, and a stable food source in arid environments. A study of *Lepidochrysops* and relatives (Espeland et al. submitted) supports the hypothesis that massive diversification occurred through the evolution of phyto-predation (6.5 Mya), as an adaptive response to the aridification. This allowed the species to survive in changing environments and disperse to drier regions.

My PhD project investigates this hypothesis on a broader scale.

A phylogeny of the genus *Lepidochrysops* and relatives (*Euchrysops* section) will be generated using a mus-eomics approach. Samples from >200 of the ~210 species are available. Distribution data is collected by georeferencing label data (>20.000 specimens). This data coupled with morphometric data will be used to test the following hypotheses: 1) Ant parasitism evolved only once in the *Euchrysops* section; 2) The high species diversity in the phyto-predacious *Lepidochrysops* is real, not a taxonomic artefact; 3) Ant parasitism is a key adaptation for survival in dry habitats; 4) Diversification of the group started in wetter, older habitats and shifted several times to available drier habitats; 5) Ant association affects niche, molecular and morphological evolution.

All collected data will be used for an integrative taxonomic revision.

Bayesian total-evidence dating under the fossilized birth–death process illuminates the age and historical biogeography of Apollo butterflies

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Fossils offer evidence for the existence of a lineage and information on the timing of the lineage's diversification, providing insights into its age, preserved traits, and geographic location. Reconstructing time-calibrated phylogenies, including both extant and fossil taxa as lineages (total-evidence dating) under the fossilized birth-death process, can provide new information on the phylogenetic position of fossil specimens, the time at which they separated from closely related species, and can impact inferences of clades' historical biogeography. Here, we focus on the Apollo butterflies (Papilionidae: Parnassiinae), whose origin has been estimated to be in the early Eocene in Central Asia. Dated to the late Oligocene (*Thaites ruminiana*) and late Miocene (*Doritites bosniackii*) in the western Palearctic, the two fossil taxa are unambiguously recovered in the subfamily Parnassiinae, thus challenging the Central Asian origin. We performed a Bayesian total-evidence dating approach based on four mitochondrial genes, one nuclear gene, and 180 morphological characters to explore the impact of dating analyses and past fossil distributions on the estimation of the evolutionary history of the group. Specifically, (i) we investigated the effect of dating phylogenies on fossil placements and node supports; (ii) we carried out total-evidence dating under the fossilized birth-death process, and examine the effect of clock partitioning (from 1 to 9 partitions) and relaxed clock models (autocorrelated, uncorrelated, and strict clocks) on the node age estimates; and (iii) we inferred parametric time-stratified biogeographic analyses with the resulting total-evidence dated tree, thus considering the past western Palearctic distributions of the two fossils.

Using Museum specimens to analyse the effect of altitude and other ecological correlates on neotropical Nymphalid butterfly wing colouration

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Butterflies exhibit some of the most beautiful, intricate, and diverse colours in nature. Wing colouration is selected for a range of functions, the most researched of which is signalling, such as conspecific signalling, camouflage, and warning/anti-predation signals. Colour can also function in thermoregulation, something that is less studied but important for fitness and survival. Knowledge on evolved methods of thermoregulation is particularly vital for ectotherms adapted to constrained biogeographical regions, such as the tropics. Understanding thermoregulation, especially in environments susceptible to global climate changes, can provide important insights into adaptation and evolution of wing morphology, and how insects may cope in changing temperatures. Whilst nymphalid butterflies are found in all zoological regions of the world, except Antarctica, 43.4% of the planet's Nymphalid species are neotropical. The neotropics are a hotspot for butterfly biodiversity, and host complex and variable geographic habitats. The variation in environment, ecology, and altitude of the neotropics, alongside the diversity of Nymphalid species, make it an ideal habitat to research the effects of environmental variation on morphology. This study looks at (1) community structure and stratification preferences of Nymphalid butterflies at different altitudes in the Andes, (2) how wing colour changes with altitude and temperature, at different forest strata, and (3) the relationship between environmental variation and structural iridescent colouration. We combine ecological modelling, phylogenetic analysis, use of natural history collections and digital imaging in colour analysis, to unpick the drivers and trends in colouration across environments.

Investigating the evolutionary relationships of the pantropical moth genus *Parasa*

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The genus *Parasa* Moore (Lepidoptera: Limacodidae) has a global disjunct distribution found throughout the tropical regions of Africa, Asia and the Americas. Members of this group display close phenotypic similarity, typically exhibiting green and brown forewing colouration. However, the pantropical distribution of a single genus is extremely unusual and some recent phylogenetic studies have uncovered the presence of multiple, similar-looking genera in other Lepidoptera, hence the need for amended taxonomy in such groups. My PhD thesis aims to utilise whole genome sequencing of *Parasa* museum specimens from across its global range to determine if a) *Parasa* is a truly pantropical genus, and how it has spread across the globe in geological time, or b) multiple genera have convergently evolved this phenotype. By answering this question, we can further our understanding of biogeographical and evolutionary processes in Lepidoptera. In addition, the use of whole genome sequencing will allow for further investigations into the gene regions controlling certain aspects of the phenotype in this group; for instance, I am interested in whether the genes controlling for the green and brown wing colouration are conserved. Finally, I also hope to compare the protein compounds involved in the toxins produced by *Parasa* caterpillars via RNA-seq, to further infer the relatedness of members of the genus. This work will bring together themes of phylogenetics, taxonomy, biogeography and toxicology to broaden our knowledge of Lepidopteran evolution across a global scale.

Vision and signalling in butterflies

The role of sound in courtship and male-male competition in *Bicyclus anynana* butterflies

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Butterflies have been shown to rely on visual signals in the form of wing patterns and on pheromones to exercise mate choice. The role of acoustic communication, however, in butterfly intraspecific communication is a largely unexplored area. Many butterfly species display ritualized courtship behaviour in the form of rapid wing flickering to attract mates. These wing flicks can lead to production of sound signals inaudible to human ears but might be audible to butterflies. African satyrine butterfly species, *Bicyclus anynana* is a polyphenic butterfly species with differences in courtship behaviour in response to rearing temperature. When compared to wet season males (reared at a temperature of 27°C), dry season males (reared at a temperature of 18°C), court less. This suggests possible seasonal differences in courtship song characteristics as well. We first demonstrate a possible role of acoustic signals in courtship in *Bicyclus anynana*, wherein we show that the males may be listening to conspecific male sounds in male-male competition. Interestingly, in presence of another male, intact males (males that can hear) were able to secure more copulations, than deaf males (whose tympanal membrane was ruptured). This suggests that exposure to competitor's courtship song might elicit a change in male's courtship behaviour and hearing might play an important role in mating. Furthermore, we will be investigating the sounds produced by wet season and dry season males during courtship. We then identify the molecular correlates of courtship sound perception in adult male and female brains.



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