7th INTERNATIONAL CONFERENCE ON THE

BIOLOGY OF BUTTERFLIES

UNIVERSITY OF TURKU, FINLAND
AUGUST 11-14, 2014

ABSTRACTS

University of Turku

Schedule

Monday August 11

08:00-08:55 Registration at conference venue (the registration desk will be open throughout the conference), downloading presentation files

08:55-09:00 Welcoming words, Niklas Wahlberg

09:00-10:00 Plenary Lecture, Ilkka Hanski

10:00-10:30 Refreshment break

10:30-12:10 Morning session, Population biology and Conservation

12:10-13:30 Lunch break, downloading presentation files

13:30-14:50 Afternoon session (Hall IX), Evolutionary Ecology

13:30-14:50 Afternoon session (Hall X), Genomic basis of adaptations

14:50-15:20 Refreshment break

15:20-16:40 Afternoon session (Hall IX), Evolutionary Ecology (continues)

15:20-16:40 Afternoon session (Hall X), Genomic basis of adaptations (continues)

16:40-17:00 Break

17:00-18:00 Afternoon session (Hall IX), Evolutionary Ecology (continues)

17:00-18:00 Afternoon session (Hall X), Genomic basis of adaptations (continues)

18:00-19:30 Get-together party at conference venue

Tuesday August 12

08:30-09:00 Downloading presentation files

09:00-10:00 Plenary Lecture, Antónia Monteiro

10:00-10:30 Refreshment break

10:30-12:10 Morning session, Genomics and Development

12:10-13:30 Lunch break, downloading presentation files

13:30-14:50 Afternoon session (Hall IX), Butterfly Movement

13:30-14:50 Afternoon session (Hall X), Sexual Selection

14:50-15:20 Refreshment break

15:20-16:40 Afternoon session (Hall IX), Butterfly Movement (continues)

15:20-16:40 Afternoon session (Hall X), Sexual Selection (continues)

16:40-17:00 Break

17:00-18:00 Afternoon session (Hall IX), Butterfly Movement (continues)

17:00-18:00 Afternoon session (Hall X), Sexual Selection (continues)

19:00-20:30 City Reception at Turku town hall

Wednesday August 13

08:30-09:00 Downloading presentation files

09:00-10:00 Plenary Lecture, Naomi Pierce

10:00-10:30 Refreshment break

10:30-12:10 Morning session, Ecology and Evolution

12:10-13:30 Lunch break, downloading presentation files

13:30-14:50 Afternoon session (Hall IX), General Butterfly Biology

13:30-14:50 Afternoon session (Hall X), Butterflies as Hosts

14:50-15:20 Refreshment break

15:20-16:40 Afternoon session (Hall IX), General Butterfly Biology (continues)

15:20-16:40 Afternoon session (Hall X), Butterflies as Hosts (continues)

16:40-17:00 Break

17:00-18:00 Poster session

19:00-23:30 Conference Banquet on Pikkupukki island, boat leaves at 19:00

Thursday August 14

08:30-09:00 Downloading presentation files

09:00-10:00 Plenary Lecture, Felix Sperling

10:00-10:30 Refreshment break

10:30-12:10 Morning session, Systematics and Diversity

12:10-13:30 Lunch break, downloading presentation files

13:30-14:50 Afternoon session (Hall IX), Evolutionary Phylogenetics

13:30-14:50 Afternoon session (Hall X), Behavioural Ecology

14:50-15:20 Refreshment break

15:20-16:40 Afternoon session (Hall IX), Evolutionary Phylogenetics (continues)

15:20-16:40 Afternoon session (Hall X), Behavioural Ecology (continues)

16:40-17:00 Break

17:00-18:00 Afternoon session (Hall IX), Evolutionary Phylogenetics (continues)

17:00-18:00 Afternoon session (Hall X), Behavioural Ecology (continues)

18:00-18:15 Final thank you and farewell!

Oral Presentation Program

Monday August 11

Plenary presentation Hall IX

09:00 Ilkka Hanski: Adaptation and survival in fragmented landscapes two decades of research on the Glanville fritillary butterfly

Morning session, Population biology and Conservation

Organized by Marjo Saastamoinen

Hall IX

10:30 Rob Wilson: Topography, microclimate and population persistence in a changing climate

10:50 Cheryl Schultz: Twenty years of ecological research on Fenders blue butterfly (*Plebejus icarioides fenderi*): Using an endangered species to advance butterfly conservation

11:10 Jenny Hodgson: Planning landscapes to enhance colonisations under climate change

11:30 Jens Roland: Hedging its bets on climate-change roulette? Rocky Mountain Apollo butterfly population dynamics

11:50 Karl Gotthard: The evolution of diapause and alternative developmental pathways in a temperate butterfly

Afternoon session, Evolutionary Ecology of butterflies — a symposium honoring the work of Christer Wiklund

Organized by Karl Gotthard and Klaus Fischer

Hall IX

13:30 Keynote presentation - Jeremy Thomas: Extreme adaptations to social parasitism by *Maculinea* butterflies and their myrmecophilous parasitoids

14:10 Hans Van Dyck: Range dynamics, climate change and the pace of life syndrome in a butterfly under niche expansion

14:30 Klaus Fischer: Dealing with climate change: limitations to plastic and genetic responses

15:20 Michael C. Singer: One butterfly, six host shifts

15:40 Magne Friberg: Decoupling of female host plant preference and offspring performance in relative specialist and generalist butterflies

16:00 Panu Välimäki: Genetic benefits may underlie female polyandry even if material benefits are involved

16:20 Andrea Grill: What prolongs a butterfly's life? Trade-offs between dormancy, fecundity and body size

17:00 Kristjan Niitepõld: The hunger games: Aging, energetics and life history under stressful conditions

17:20 Elizabeth Long: Introducing a female-limited polymorphic mimicry system in North American checkerspots

17:40 Toomas Esperk: Between-generation size differences in butterflies are not caused by environmental effects on larval growth performance

Afternoon session, Genomic basis of adaptations

Organized by Chris Wheat and Patrícia Beldade

Hall X

13:30 Keynote presentation - Mathieu Joron: When adaptation brings its load: speciation vs. polymorphism in a butterfly genome

14:10 Casper Breuker: Maternal regulation of early embryogenesis in the Speckled Wood butterfly, *Pararge aegeria*

14:30 Jason Hill: I genomics: how to generate a high quality genome in less than one year and its use in studying the evolutionary dynamics of host plant detoxification.

15:20 Juan Galarza: The fitness effects of phenotypic and genomic plasticity across life-stages

15:40 Caroline Nieberding: Transcriptomics of olfactory communication mediated by sex pheromones in *Bicyclus anynana*

16:00 Peter Pruisscher: Genetic variation in diapause-related genes in *Pararge aegeria*

16:20 Ilik Saccheri: Multiple origins of industrial melanism in the peppered moth

17:00 Vicencio Oostra: Effects of predictable and unpredictable juvenile conditions on adult life history: an RNA seq analysis in a butterfly

17:20 Alok Arun: The pheromone biosynthesis activating neuropeptide (PBAN): a conserved molecular mechanism for regulating sex pheromone production across Lepidoptera?

17:40 Violaine Llaurens: Evolution of dominance in a polymorphic butterfly involved in Müllerian mimicry

Tuesday August 12

Plenary presentation Hall IX

09:00 Antónia Monteiro: The origin and evolution of novel gene regulatory networks

Morning session, Genomics and Development

Organized by Patrícia Beldade and Chris Wheat

Hall IX

10:30 Chris Jiggins: The origins of dennis: Evolutionary innovation through modular regulation of an input-output gene

10:50 Peter Holland: New genes and new microRNAs in lepidopteran evolution

11:10 Marcus Kronforst: doublesex is a mimicry supergene

11:30 Virpi Ahola: The Glanville fritillary genome retains an ancient karyotype and reveals exceptional chromosome stability in Lepidoptera

11:50 Chris Wheat: Genomics and the evolution of central metabolism in butterflies

Afternoon session, The ecology of butterfly movement

Organized by Cheryl Schultz

Hall IX

13:30 Keynote presentation - Carol Boggs: Butterfly movement: when to go and when to stay

14:10 Diane Debinski: Species traits as filters of climate-induced range expansion

14:30 Elizabeth Crone: Does habitat use reflect habitat quality? A comparison of three butterfly species in restored and remnant habitat patches

15:20 Nusha Keyghobadi: Connectivity and the genetic effects of a population collapse

15:40 Anniina Mattila: Flight and dispersal in the Glanville fritillary butterfly: genes, physiology and environmental effects

16:00 Jorge L. León-Cortés: The population dispersal and conservation needs of montane endemic butterflies in Southern Mexico

16:20 Leone Brown: Movement-based approaches for estimating population growth rates and critical minimum patch size

17:00 Mikko Kuussaari: Higher mobility of butterflies than moths connected to habitat suitability and body size in a release experiment

17:20 Nicolas Schtickzelle: Multicausality of dispersal variation in a butterfly metapopulation

17:40 Anuj Jain: Importance of dispersal and metapopulation dynamics in tropical butterfly conservation

Afternoon session, Integrating Pre- and Post-Copulatory Sexual Selection in the Lepidoptera: New Insights and Future Directions

Organized by Nathan Morehouse, Christer Wiklund and James Walters

Hall X

13:30 Keynote presentation - Ronald Rutowski: Sexual selection, Darwin, and butterflies: Then and now

14:10 Carlos Cordero: Sexual coevolution and Lepidoptera genitalia

14:30 Erica Westerman: Butterfly mate preference learning: Sexually dimorphic learning, learning biases, and multimodal signaling

15:20 Zenobia Lewis: The interplay between different stages of reproduction in *Plodia interpunctella*

15:40 Bárbara Huber: Coexisting closely related species under the same disguise: insights into species recognition issues between *Heliconius hecale* and *H. ismenius*

16:00 Jacobus de Roode: Testing for cryptic female choice in monarch butterflies

16:20 James Walters: Proteomic analysis of eupyrene and apyrene sperm in Monarch butterflies

17:00 Nathan Morehouse: Male ejaculates and female reproductive adaptations in the polyandrous butterfly *Pieris rapae*

17:20 Oskar Brattström: Evolutionary patterns of male sexual characters across Mycalesina butterflies

17:40 Richard Merrill: Sexual conflict and the evolution of novel warning patterns in a Mllerian mimic

Wednesday August 13

Plenary presentation Hall IX

09:00 Naomi Pierce: Diversification of the Lycaenidae: key characters involved in speeding up and slowing down

Morning session, Ecology and Evolution

Organized by André Freitas

Hall IX

10:30 Sören Nylin: Evolution of host plant use in butterflies

10:50 James Fordyce: A tale of two communities: temporal and spatial turnover in vertically stratified tropical fruit-feeding Nymphalid communities

11:10 Paul Brakefield: Thoughts on the contributions of research on the biology of butterflies to the biosciences

11:30 André Freitas: When canopy comes to the ground: effects of forest disturbance on community structure of tropical butterflies

11:50 Krushnamegh Kunte: Ecological drivers of sexual dimorphism and polymorphism in mimetic butterflies

Afternoon session, General butterfly biology

Organized by Niklas Wahlberg and Carlos Peña

Hall IX

13:30 Christopher Hamm: What is the Mithcell's satyr butterfly? Contemporary approaches to an old question

13:50 Vlad Dincă: *Leptidea* Wood White butterflies as an emerging model to study speciation

14:10 José R. Ferrer-Paris: The assessment of macroecological patterns in butterfly-hostplant associations at a global scale

14:30 Juha Pöyry: A positive relationship between host plant nitrogen content and size of butterflies and moths enhances the observed opposite population trends of species

15:20 Freerk Molleman: The Rhythm of Africa: dynamics of a fruit-feeding butterfly community

15:40 Blanca Huertas:Back to basics: The role of systematics in conservation assessments of butterflies in the Neotropics

16:00 Raluca Voda: Biodiversity loss in the Maltese islands hits relictual species

16:20 Toomas Tammaru: The gradation from capital to income breeding as an informative way to ordinate lepidopteran life histories

Hall X

16:20 Vladimir Lukhtanov: Homoploid hybrid speciation and genome evolution via chromosome sorting

Afternoon session, Butterflies as hosts

Organized by Anne Duplouy

Hall X

13:30 Keynote presentation - Jaap de Roode: Parasite infections, toxic milkweeds and medication behavior in monarch butterflies

14:10 Emily Hornett: The genomic impact of intense selection in a butterfly

14:30 Melanie Gibbs: Maternal effects and offspring immune defence in *Pararge aegeria*: implications for dispersal in fragmented landscapes

15:20 Martinus Huigens: Exploitation of butterfly anti-sex pheromones by tiny hitch-hiking parasitic wasps

15:40 Saskya van Nouhuys: Variation in a host-parasitoid interaction across independent populations

16:00 Anne Duplouy: Inbreeding versus symbiont-induced incompatibility: which of the two for a better conservation strategy?

Thursday August 14

Plenary presentation Hall IX

09:00 Felix Sperling: Butterfly biodiversity and operational species

Morning session, Systematics and Diversity

Organized by Niklas Wahlberg

Hall IX

10:30 Carla Penz: *Morpho*, *Bia* and Biogeography (Nymphalidae, Satyrinae)

10:50 Fabien Condamine: Testing the Red Queen and the Court Jester Hypotheses: The Role of Ecological and Evolutionary Determinants in the Radiation of Apollo Butterflies (Papilionidae: Parnassiinae)

11:10 Marianne Elias: Systematics, ecology and diversity of ithomiine butterflies

11:30 Roger Vila: Cryptic matters: the relevance of hidden butterfly diversity

11:50 Akito Kawahara: Phylogenomics using nearly 2700 genes provides strong evidence for relationships of butterflies and moths

Afternoon session, Evolutionary phylogenetics and Lepidoptera biodiversity: diversification, adapations and community ecology

Organized by Marianne Elias and Karina Lucas da Silva-Brandão

Hall IX

13:30 Keynote presentation - Niklas Janz: What is host range? (happy meetings between phylogenetic and experimental studies)

14:10 Robin van Velzen: Asynchronous divergence of *Cymothoe* forest butterflies and their *Rinorea* host plants in tropical Africa

14:30 Kwaku Aduse-Poku: Spectacular radiation of Mycalesina butterflies in the old world tropics

15:20 Jessie Santos: Effect of vertical stratification in the phylogenetic structure of a fruit feeding butterflies community of an Atlantic Forest, Brazil

15:40 Nicholas Chazot: Diversification in the Neotropics: insights from Ithomiini butterflies.

16:00 Ivonne J. Garzón-Orduña: An alternative, plant-based time-tree implies conflicting dates for the diversification of ithomiine butterflies (Lepidoptera: Nymphalidae: Danainae)

16:20 David Lohman: Evolution and biogeography of Batesian mimetic diversity in *Elymnias* butterflies (Nymphalidae: Satyrinae)

17:00 Erik van Bergen: Natures ecological recorders - stable isotopes reveal patterns of ecological diversification in Mycalesine butterflies

17:20 Marianne Espeland: Higher-level phylogeny and biogeography of the Riodinidae

17:40 April Dinwiddie: Developmental and evolutionary consequences within the lepidopteran wing scale morphospace

Afternoon session, Behavioural and neurological aspects of ecological adaptation and speciation

Organized by Stephen Montgomery and Richard Merrill

Hall X

13:30 Keynote presentation - Steven M. Reppert: Neuroethology of monarch butterfly migration

14:10 Erica L. Westerman: Perceptual Biases and Visual Attraction: The Role of Perception in Reproductive Isolation

14:30 Brett M. Seymoure: *Heliconius* mimicry rings in a new light: Light environment and signaling

15:20 Emilie Snell-Rood: Nutrition as a constraint on brain and life history evolution: a comparative study across butterflies

15:40 Maaike de Jong: Ecological genetics of life history and host plant adaptation in the Glanville fritillary butterfly

16:00 Alexander Schäpers: Specialist and generalist oviposition strategies in butterflies: maternal care or precocious young?

16:20 Carita Lindstedt: Genetic and environmental constraints on protectiveness of coloration: a genetic trade-off between efficacy of adult and larval warning coloration in an aposematic moth

17:00 Kimberly Pegram: Relative effectiveness of blue and orange warning colors at deterring naïve avian predators

17:20 Rachel Olzer: Heritability of Bright Coloration in the Pipevine Swallowtail (*Battus philenor*)

17:40 Takeuchi Tsuyoshi: The erroneous courtship hypothesis: do butterflies really engage in aerial wars of attrition?

MONDAY 11.8.2014

MORNING SESSION: Population Biology and Conservation

PLENARY 09:00-10:00

Adaptation and survival in fragmented landscapes – two decades of research on the Glanville fritillary butterfly

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Research on the Glanville fritillary metapopulation in a network of 4,000 small meadows in the Åland Islands in SW Finland has contributed many insights into ecology, genetics and evolution of species living in fragmented landscapes. The recently sequenced genome of the butterfly and a high-density linkage map offer new opportunities to integrate molecular, genomic and ecological research. There has been no long-term trend in the size of the metapopulation, but increasing frequency of unfavorable (hot and dry) summers has increased the spatial synchrony of dynamics, with potentially adverse consequences for long-term survival. The demographic extinction-colonization dynamics are closely coupled with microevolutionary changes in several traits, including flight metabolism and dispersal, which are associated with *Pgi* polymorphism. Glanville fritillary populations from fragmented versus continuous landscapes in northern Europe exhibit significant differences in many life history traits, e.g. butterflies from fragmented landscapes have greater dispersal capacity than butterflies from continuous landscapes. In a common garden experiment, a large number of genes (1,841) were differentially expressed between the landscape types. Genes that were more highly expressed in butterflies from newly-established than old local populations within a fragmented landscape were more highly expressed, at the landscape level, in fragmented than continuous landscapes. This result suggests that recurrent extinctions and re-colonizations in fragmented landscapes select for a specific expression profile. Genes that were significantly up-regulated following an experimental flight treatment had higher basal expression in fragmented landscapes, indicating that these butterflies are genetically primed for frequent flight. I conclude that differences in gene expression between the landscape types reflect genomic adaptations to habitat fragmentation.

ORAL 10:30-10:50

Topography, microclimate and population persistence in a changing climate

Wilson, Robert(1), Bennie, Jonathan(1), Suggitt, Andrew(1), Lawson, Callum(2), Maclean, Ilya(1)
(1)University of Exeter, UK (2)Netherlands Institute of Ecology, The Netherlands
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Recently observed shifts in the distributions of many species to higher latitudes and elevations provide empirical support for predictions of climate change as a threat to biodiversity. However, the ecological responses of butterflies to climate change depend on complex patterns of variability in weather and local microclimate that overlay global increases in mean temperature. Here we show for the UK distribution of the skipper butterfly *Hesperia comma* how variation in microclimate from 1982 to 2009 influenced population and metapopulation dynamics, and hence spatial and temporal patterns of range contraction and expansion. Across a wider range of butterflies and other taxa in the UK, the effects of climate warming on patterns of persistence versus extinction at the 10 km scale are modified by spatial variation in topoclimate. These results suggest that variation in microclimate created by topography could buffer populations of species against climate variability, presenting the opportunity to adapt conservation to climate change by targeting topographically heterogeneous landscapes.

ORAL 10:50-11:10

Twenty years of ecological research on Fender's blue butterfly (*Plebejus icarioides fenderi*): Using an endangered species to advance butterfly conservation

Schultz, Cheryl(1), Crone, Elizabeth E.(2)
(1)Washington State University, USA (2)Tufts University, USA
schultzc@vancouver.wsu.edu

In this talk I offer highlights from twenty years of ecological research to advance butterfly conservation. In 1993 Fender's blue butterfly (*Plebejus icarioides fenderi*) was a newly rediscovered species in remnant prairies in Oregon's Willamette Valley. Over the next two decades, new populations were found, threats enumerated, Fender's blue listed as "endangered," and a proliferation of efforts to recover the butterfly and its habitat across a largely private landscape ensued. As questions emerged, we developed ecological tools to address questions of conservation concern for Fender's blue and to provide guidance on how to generalize lessons learned across taxa. I highlight a few of these tools and lessons. First, I highlight rules of thumb that emerge from our work – stepping stones, dispersal distance and minimum patch size. Second, I touch on use of estimated minimum abundance, EMA, to guide recovery planning. Third, I discuss tools for landscape planning that integrate dispersal and demography – butterfly networks in the context of restoration and management. Lessons from these studies lead to specific recovery criteria that are being implemented by US Fish and Wildlife Service and provide guidance to land management agencies on-the-ground

ORAL 11:10-11:30

Planning landscapes to enhance colonisations under climate change

Hodgson, Jenny(1)

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The issue of climate change, among others, has prompted conservationists to focus on planning for entire landscapes, rather than individual protected areas. There is also a CBD target to restore 15% of degraded ecosystems worldwide. This offers great opportunities to mould the landscapes of the future to minimise biodiversity loss, but is also a formidable challenge to our ecological understanding. I have argued that increasing the area and quality of habitats should be a robust way to facilitate range shifting by species, and that optimising the spatial arrangement of habitat is a secondary concern. In support of this, empirical work has recently shown that existing protected areas are facilitating the extension of northern range margins for a wide range of UK species, including butterflies. Furthermore, for the butterfly *Hesperia comma*, it has been shown that sites which are actively managed for conservation are much more likely to be colonised than unmanaged sites of similar vegetation type, but that less targeted agri-environment scheme management has no detectable effect. At the same time, however, my theoretical work has shown that, when habitat is very scarce and metapopulation-like dynamics occur, then the speed of range expansion is highly sensitive to the spatial arrangement of habitat. This could make it more difficult to find a habitat restoration strategy that works for a broad range of species. But one way forward is to ask, on a continental scale, is it possible for species to bypass heavily fragmented landscapes as they shift? If it is, then I would argue that focusing on habitat availability and quality in the less fragmented landscapes is still a good strategy. If it is not, then actions that link currently isolated landscapes should be considered, but planning should be on much larger scales than most current conceptions of "ecological networks".

ORAL 11:30-11:50

Hedging its bets on climate-change roulette? Rocky Mountain Apollo butterfly population dynamics

Roland, Jens[1]
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Alpine areas are characterized by highly variable temporal and spatial patterns of weather and climate. As such, alpine organisms may be particularly vulnerable to effects of climate change. We use 20 years of population estimates for 21 populations of alpine *Parnassius smintheus* butterflies, in combination with mixed-effects models and regression trees in an effort to identify interactions among weather variables affecting population growth. Winter weather is far more important than is summer. A combination of November minimum temperatures and the timing of snow melt in spring explain much of the variation in population change, suggesting a combined importance of snow and temperature for the over-wintering egg. Density-dependent effects of population growth are only manifest in the more benign years. Surprisingly, cold summers appear to promote population growth. Patterns of complex interaction, and some seemingly paradoxical results are discussed in light of life-history traits of *P. smintheus* that mitigate fine-scale spatial and short-term temporal variability in weather, and which may buffer alpine butterfly populations from expected impact of climate change.

ORAL 11:50-12:10

The evolution of diapause and alternative developmental pathways in a temperate butterfly

Gotthard, Karl[1]
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The ability of many temperate insects to have more than one generation per year is due to the plastic induction of alternative developmental pathways - leading to either direct development during summer or diapause development during winter. Typically, the number of generations produced within a population decrease with increasing latitude and the developmental threshold determining which pathway to follow is predicted to show a latitudinal cline due to local adaptation to season length. Consequently, when moving north along a latitudinal cline, populations will ultimately become univoltine and only produce one diapausing generation per year. This means that selection on developmental regulation of the direct pathway will be relaxed. I will present results showing how the photoperiodic threshold determining the pathway decision in the butterfly *Pararge aegeria* is locally adapted to season length. Moreover, I will explore footprints of relaxed selection on the developmental regulation of life history traits and sexual dimorphism when expressed in the direct development pathway. The results suggest that populations experiencing relaxed selection on the direct pathway show less pronounced differences between pathways in several life history phenotypes. In particular, relaxed selection on direct development was associated with a disruption of protandry (earlier emergence of adult males) expressed as sexual dimorphism in larval development time and growth rate. This suggests that ongoing selection in southern populations is necessary for upholding this type of "fine tuning" of alternative developmental regulation.

AFTERNOON SESSION: Evolutionary Ecology

KEYNOTE 13:30-14:10

Extreme adaptations to social parasitism by *Maculinea* butterflies and their myrmecophilous parasitoids

Thomas, Jeremy A.[1]
[1]University of Oxford, UK

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The larvae of *Maculinea* butterflies have evolved a suite of mechanisms that enable them to infiltrate, inhabit, and exploit the rich resources found within *Myrmica* ant colonies. Most involve the corruption, by mimicry, of the honest signals that enable members of their host ant colony to function cohesively. Initial penetration is achieved by secreting a simple cocktail of genus-specific mimetic hydrocarbons that induces the undiscerning foragers of any *Myrmica* species to carry young larvae into their nests. Once there, the larva will either secrete a more complex mixture of chemicals that more closely mimics the recognition signals of a single (primary host) species of *Myrmica* or, if adopted by other species of *Myrmica*, it supresses its own secretions and instead relies on acquiring the odour of its host through physical contact, a strategy that seldom results in survival over the one or two-year larval growth phase. After a larva is accepted as a colony member by worker ants, it elevates its status within the society by mimicking the distinctive acoustical signals made by adult queen ants, thereby receiving priority 'royal' treatment from the workers. Two strategies for exploiting ant resources evolved early among the *Maculinea*: predation and cuckoo-feeding. The latter, involving direct feeding by workers, is more efficient and results in larger, more stable butterfly populations: a cost is more extreme host specificity in most populations of cuckoo-feeding species, which have radiated to display amplified host-shifts across Europe. All types of *Maculinea* are themselves parasitized by ichneumonid parasitoids, which rely on agonistic allomones and armour to walk safely within ant nests. Most forms of *Maculinea* are globally or regionally threatened. Only through understanding their adaptations and ecology have biologists managed to conserve certain populations, exemplified by the restoration of *M. arion* to the UK.

ORAL 14:10-14:30

Range dynamics, climate change and the pace of life syndrome in a butterfly under niche expansion

Van Dyck, Hans(1), Holveck, Marie-Jeanne(1)
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Many species, including butterflies, respond to current climate change by range shifts. Newly colonized populations may differ in phenotypic and genotypic traits from well-established populations at the core of the range. Comparisons of traits between edge and core populations have contributed significantly to our understanding of range dynamics under climate change. However, the binary nature of such comparisons ignores the dynamics within the core of the range. At the core, populations may be invaded, and even replaced, by different ecotypes or by southern genotypes of different life style revealing considerable within-range dynamics. Temperature-related physiological profiles may vary along latitudinal gradients resulting in higher pace of life under more northern environmental conditions. Within this context, we will present the results of our work on speckled woods (*Pararge aegeria* L.). In the laboratory, we simulated a split-brood reciprocal transplant experiment between two latitudinal zones by rearing offspring of females at the temperature of the northern latitude (Belgium) and of the southern latitude (France). At both latitudes individuals of woodland landscape origin (ancestral habitat) and of agricultural landscape origin (recent habitat) were used. Our earlier work revealed differences in functional traits between both ecotypes (e.g. behaviour, morphology, temperature-related fecundity); woodlands are humid, cooler and thermally buffered habitats, whereas the habitat in agricultural landscape is drier, warmer, but also more variable, and much more fragmented as well. After larval development under either northern or southern thermal regime, we compared their pace of life (resting metabolic rate) at the adult stage relative to latitude, ecotype and sex. We also studied flight performance (flight mill test combined with respirometry) and functional morphology. Our results on differential responses and different degrees of plasticity among latitudes, ecotypes and sexes will be discussed within the framework of range dynamics and eco-evo-devo responses under global change.

ORAL 14:30-14:50

Dealing with climate change: limitations to plastic and genetic responses

Fischer, Klaus(1)
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Rising temperatures, caused by anthropogenic climate change, are considered a major threat to biodiversity, although all organisms possess mechanisms to deal with environmental variation. Due to the concomitant plastic and genetic capacities, predicting the responses of individual species to climate change is challenging. Predictions are, amongst others, complicated by the following issues. First, while slightly increased mean temperatures *per se* are unlikely to exert strong negative effects on biodiversity, the high temperatures associated with heat waves may exceed the physiological tolerances of many species. Second, temperature effects on biotic interactions, e.g. indirect effects mediated *via* thermal effects on host plant quality, are hitherto poorly understood. Third, the human-induced loss of natural habitats often reduces population size and concomitantly genetic diversity through inbreeding or genetic drift, which may in turn feedback on a species' ability to cope with stressful conditions. While in general the detrimental impact of e.g. inbreeding has often been documented, its consequences for evolutionary potential and the ability to cope with temperature stress are only poorly understood. I will therefore focus on the above issues, namely heat waves, indirect thermal effects, and the interference between inbreeding and stress resistance, using different butterfly species and populations as study systems.

ORAL 15:20-15:40

One butterfly, six host shifts

Singer, Michael C.(1)
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Diets of around 50 populations of the North American butterfly *Euphydryas editha* have been observed over a period of 25–45 years. Most have remained constant, but six populations have undertaken host shifts, independently of each other. Three of the shifts have been ecological, caused by year-to-year changes in host acceptability or availability and not requiring evolutionary change of either plant or insect. The other three shifts have involved evolution of oviposition preference of the butterflies, with dramatic changes of host affiliation. In each case there was a surge to add a novel host to the diet. In one case this diet expansion was caused by anthropogenic alteration of host suitability; a second was caused by natural population extinction and recolonization; and the third by anthropogenic introduction of an exotic host. In all three cases the novel host became the principal host of the butterfly population, receiving more eggs than any other host. However, by 2014 all three evolutionary events had reverted to their starting points; the novel hosts had all been completely eliminated from the diets of the three rapidly-evolving populations. In the first case this reversion resulted from a change in natural selection on the insects following succession; in the second, it resulted from response by immigrant insects to unchanging natural selection. The third reversion was caused by population extinction and recolonization from a source resembling the starting population.

ORAL 15:40-16:00

Decoupling of female host plant preference and offspring performance in relative specialist and generalist butterflies

Friberg, Magne(1), Posledovich, Diana(2), Wiklund, Christer(2)
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The classic 'preference-performance' hypothesis suggests that the butterfly host plant range is ultimately limited by genetic trade-offs acting on growing larvae. These trade-offs are believed to result in host plant specialization, because both females and offspring would ultimately be favored by utilizing only the most suitable host(s). Historically, this hypothesis has received only mixed empirical support, and the difficulty of obtaining supporting evidence has been attributed to several ecological factors. Here, we develop a comparative experimental approach to circumvent these problems and present the results of a suite of laboratory experiments on two sympatric and syntopic populations of the closely related butterflies *Pieris napi* and *Pieris rapae* (Pieridae). These populations show variation in their level of host plant specialization. We tested three specific predictions from the preference-performance hypothesis, and although our results showed the expected pattern – a positive correlation between female preference and offspring performance – we could not attribute this pattern to any larval feeding trade-offs. First, the link between female host preference and offspring performance was not significantly stronger in the relative host plant specialist. Second, the offspring of the host plant specialist did not outperform the offspring of the generalist species on the former's most preferred host plant species. Finally, the generalist did not show higher survival or consistently higher growth rates than the specialist on the less preferred plants. Hence, the data suggest that the variation in female host preferences does not correspond to host plant related trade-offs acting on the larval offspring, but that the preference and performance traits evolve as largely separated units.

ORAL 16:00-16:20

Genetic benefits may underlie female polyandry even if material benefits are involved

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Bateman's seminal contribution in the 1940s states that females gain only little from mating with several males or repeatedly with a single partner. In addition to a sexually antagonistic explanation (convenience polyandry), the suggested explanations on the origin of taxonomically widespread polyandry can be divided into two categories: genetic or material benefits. In species where males provide their partners with nutritious substances at mating, material benefits of polyandry seem of profound importance. Yet, the evolution of voluntary nuptial provisioning is only possible under extraordinary conditions, whereas possibilities for genetic benefits are inherently present every time females engage in mating with multiple partners. Concentrating solely on material benefits may, thus, reveal the maintenance of polyandry, but easily ignore its evolutionary origin. A theory predicts polyandrous mating system with decreasing variation in genetic quality among males but with increasing probability of genetic incompatibilities between the mating pair. A form of genetic incompatibility is inbreeding depression, which stands for a relative decline in fitness in offspring of related parents. Inbreeding tolerance is expected to be low and selection for inbreeding avoidance intense when both sexes invest substantially in reproduction such as in nuptial gift-giving butterflies. We explored implications of inbreeding in the green-veined white butterfly (*Pieris napi*) with substantial material benefits of multiple mating. Compared to the outbred ones, partially inbred eggs and neonate larvae had a lower hatching success and a lower survival until pupation, respectively. Indicative of a post-copulatory inbreeding avoidance, female remating intervals decreased following incestuous matings. In addition, the relative decrease in remating interval and female egg-laying rate following mating with a close kin correlated positively with the genotype-specific inbreeding depression coefficients. We conclude that incest-induced shift in the phenotype may contribute to the evolution of female mating rates although alternative explanations in addition to material benefits have rarely been invoked when nuptial feeding is involved.

ORAL 16:20-16:40

What prolongs a butterfly's life? Trade-offs between dormancy, fecundity and body size

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Trade-off theory is built on the concept that every organism has to balance between the optimal investment of resources in growth, reproduction and self-maintenance. In butterflies, life span often increases only at the expense of fecundity. Previous experiments in adult Meadow Brown butterflies of the genus *Maniola* have shown large intraspecific differences in life span, related to geographic provenance and summer dormancy. Here, we experimentally induced summer dormancy and hence prolonged the butterflies' life in order to study potential trade-offs of such an artificially prolonged life. We were able to modulate longevity in female adult butterflies through photoperiodically-induced summer dormancy, thereby more than doubling their natural life span, up to 246 days. Body size did not have any significant effect related to longevity, and only influenced fecundity in the smaller species. Our data show that a prolonged adult life span does not necessarily convey trade-offs in fecundity. This contrasts the mostly *Drosophila*-based findings that selection for longevity is correlated with reduction in fecundity. We conclude that Meadow Brown butterflies have an extraordinary plasticity in aging, which allows them to extend life span in response to environmental conditions. As income breeders they seem to have found a solution to the trade-off between reproduction and life span without incurring severe fitness costs.

ORAL 17:00-17:20

The hunger games: Aging, energetics and life history under stressful conditions

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Environmental variation can affect resource availability. How do butterflies respond to situations where resources are scarce or scattered across the landscape? We assessed experimentally the effects of limited nectar availability and increased flight on butterfly energetics and life history. We also examined if stress affected the rate of metabolic senescence. In the first experiment, we subjected two North American butterflies, *Speyeria mormonia* and *Colias eurytheme*, to adult dietary restriction (DR). We reduced the sugar-water intake of females in the DR treatment by 50% compared to control females. We found that DR resulted in lower physiological activity: slower resting metabolic rate and dramatically reduced fecundity. *S. mormonia* experienced a stronger decrease in reproductive output than *C. eurytheme*, possibly because of differences in egg composition. Food-restricted females did not invest in resource storage as they were lighter than control females. Interestingly, flight metabolic rate was not affected by DR, which highlights the importance of flight for butterflies. Flight metabolic rate decreased with age, but the rate of senescence did not differ between the treatments. In contrast, resting metabolic rate decreased strongly with age in control females but not in DR females, who showed stable, chronically reduced resting metabolic rate. In our second experiment, we manipulated energy consumption by forcing female *Speyeria mormonia* butterflies to fly in a cage every morning throughout their lives. Experimentally increased flight had no effect on body mass or fecundity. We found that the main reason for this was increased food intake, as food was provided *ad libitum*. Increased flight elevated resting metabolic rate and accelerated senescence in flight metabolic rate. Taken together, our results indicate that DR can have direct effects on population dynamics. *Speyeria* could compensate for increased energy consumption but flight itself is stressful. If both stressors occur simultaneously, more dramatic effects are expected.

ORAL 17:20-17:40

Introducing a female-limited polymorphic mimicry system in North American checkerspots

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Checkerspot butterflies in the genera *Euphydryas* and *Chlosyne* exhibit phenotypic polymorphisms along a well-defined geographical and elevational gradient in California (USA). The patterns of phenotypic variation in *Euphydryas chalcedona* and *Chlosyne palla* suggest a mimetic relationship; the specific patterns of variation in *C. palla* suggest a female-limited polymorphic mimicry system. However, the existence of polymorphic models is counter to predictions of mimicry theory. Using a generalized avian predator, black female *C. palla* is shown to be a female-limited polymorphic mimic (FPM) of black *E. chalcedona*, whereas both species are palatable in the red form. We also demonstrate that a related species, *C. hoffmanni*, is palatable to a generalized avian predator. We then used a long-term dataset to test theories pertaining to the flight time and abundance of Batesian and FPM systems in California. We found evidence of negative frequency-dependent selection in Batesian mimics, but not in FPM systems. We also found evidence for the model-first emergence hypothesis in Batesian, but not FPM, systems.

ORAL 17:40-18:00

Between-generation size differences in butterflies are not caused by environmental effects on larval growth performance

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Seasonal generations of short-lived organisms often differ in their morphological, behavioural and life history traits, including body size. These differences may be either due to immediate effects of a seasonally variable environment on organisms (responsive plasticity) or rely on presumably adaptive responses of organisms to cues signaling forthcoming seasonal changes (anticipatory plasticity). When directly developing individuals of insects are larger than their diapausing/overwintering conspecifics, the pattern is typically ascribed as responsive plasticity in larval growth. We tested this hypothesis using the papilionid *Iphiclides podalirius* and the nymphalid *Araschnia levana* as models. In laboratory experiments, we demonstrated that seasonal differences in food quality could not explain the observed size difference between directly developing and overwintering individuals. Similarly, the size differences are not likely to be explained by the immediate effects of ambient temperature and photoperiod on larval growth. The qualitative pattern of natural size differences between the directly developing and overwintering individuals could be reproduced in the laboratory as a response to photoperiod, indicating an anticipatory character of the response. Nevertheless, the species differed in respect of when and how during larval ontogeny directly developing individuals achieved larger size than their overwintering conspecifics. In both species the size difference between directly developing and overwintering individuals was more pronounced in the adult than the pupal stage, suggesting that weight loss during overwintering may play a role in determining between-generation size differences in studied species. Our results indicate that between-generation size differences in the studied butterflies are not caused by immediate effects of environmental factors on larval growth, but, at least partly, are the outcome of anticipatory responses and thus likely have an adaptive explanation. It remains to be studied whether the seasonal differences in adult size are adaptive *per se*, or if they constitute by-product of processes related to the diapause.

AFTERNOON SESSION: Genomics of adaptations

KEYNOTE 13:30-14:10

When adaptation brings its load: speciation vs. polymorphism in a butterfly genome

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Müllerian mimicry in butterflies is a well-known example of convergence favouring the fixation of alleles corresponding to local co-mimics, and acting against polymorphism. Assortative mating on mimicry signals promotes cladogenesis and speciation. However, some species engage in mimicry of multiple models within a locality, and simultaneously maintain multiple adaptive morphologies. In *Heliconius numata*, mimicry polymorphism is maintained by a supergene, a single major effect locus with alleles coding for distinct mimetic forms, and characterised by multiple polymorphic inversions suppressing local recombination. However, the forces driving polymorphism in association with inversions are still largely unknown. We showed that the classic model of wing pattern-based assortative mating does not operate in this species. Instead, we report the first mention of negative assortative mating based on wing phenotype (and inversion genotype), favouring heteromorphic pairings in a butterfly. Dominant and recessive forms show preference against their own patterns; intermediate dominants do not show any preference. This pattern is consistent with genotypic frequencies found in natural populations, notably the rarity of dominant homozygotes, and might suggest that deleterious mutations are linked to inversions. In contrast, predation experiments using butterfly dummies showed that the recessive morph was more strongly attacked, consistent with a relative scarcity of their co-mimics in the local habitats, which would promote disassortative mating associated with this morph. Genome-wide demographic inferences and population structure based on re-sequenced genomes reveal strong demographics and a relative lack of population differentiation, distinct from the pattern seen in other species. We hypothesise that disassortative mating at the wing pattern locus, often involved in mate recognition in *Heliconius*, may strongly act here against cladogenesis. Our data suggests that adaptive polymorphism results from a balance between frequency dependence favouring the commonest wing patterns in a variable habitat, and negative frequency-dependence on inversions carrying deleterious mutations

ORAL 14:10-14:30

Maternal regulation of early embryogenesis in the Speckled Wood butterfly, *Pararge aegeria*

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Using the Speckled Wood butterfly *Pararge aegeria* as the model species, we conducted the first comprehensive investigation of all the genes essential for butterfly oogenesis and in particular those used as maternal effect genes regulating embryonic development and extra-embryonic

tissue formation. High throughput RNA sequencing of the ovaries and freshly laid eggs was used to identify all of these genes, and a combination of techniques (e.g. RT-PCR and in-situ hybridisation) was used to characterise the spatio-temporal expression patterns of a number of key genes involved in regulating 1) offspring body axis specification, 2) establishment of the next germ cell line, and 3) extraembryonic tissue specification. Although *P. aegeria* expresses a large number of genes orthologous to those expressed by other insects during oogenesis, the results clearly show that butterflies are highly divergent for these three developmental processes as well as in oocyte production and overall egg provisioning. Furthermore, *P. aegeria*, and butterflies in general, possess duplications of a number of developmental genes such as nanos and zen (Shx genes) with possible subfunctionalisation. Putative functions of these genes will be discussed. Possible reasons for the divergent nature of butterfly oogenesis and early embryogenesis will be discussed within an evolutionary ecological context.

ORAL 14:30-14:50

Pieris napi genomics: how to generate a high quality genome in less than one year and its use in studying the evolutionary dynamics of host plant detoxification

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Within the last year we have used a variety of techniques to assemble and annotate the *Pieris napi* genome (e.g. Illumina and PacBio data for contig assembly and scaffolding, the integration of QTL mapping for scaffold ordering on chromosomes, automated and manual curation of annotations based on RNA-sequencing and protein homology). Using our high quality genome, here I report the use of Pool-Seq data to investigate the evolutionary dynamics of the nitrile-specifier protein (NSP) in the genus *Pieris*. NSP has a central role in detoxifying Brassicales plant chemical defenses (glucosinolates) and is known to be exceptionally diverse within and between populations of *Pieris*. Here we report on the extent of that diversity within and among a range of species.

ORAL 15:20-15:40

The fitness effects of phenotypic and genomic plasticity across life-stages

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Many Lepidopterans advertise unpalatability through warning signals in the form of colour and shape. A trade-off is inevitably faced to either allocate resources to signal efficiency or to other processes such as thermoregulation. It is unclear, however, if allocating resources to warning signals at the larval stage can transfer fitness advantages to the adult stage. Here we address this question by rearing full-sib Tiger moth larvae (*Parasemia plantaginis*) in different temperature treatments and follow their warning signal development from the larval stage until adulthood. Gene expression profiles were obtained for each larval developmental stage and compared between treatments. At the adult stage, we tested for fitness differences as measured by heating time and metabolic rates. We found 57 differentially expressed genes between the treatments involved mainly in immune response, cuticular protein formation, and thermal tolerance. Moreover, trypsin-like protein, lysosomal acid, and phosphoglucose isomerase were positively associated with increases/decreases of the warning signal. No clear pattern, however, was observed in melanin-related genes. Nonetheless, larvae reared at low temperatures decreased their warning signal size along its development, producing more melanised body segments. Adults reared in higher temperatures showed higher amount of melanin in their thorax, but equal amount of melanin in their fore and hind wings than adults reared in low temperatures. Finally, adults reared at high temperature showed faster heating rate, but no differences in metabolic rates were observed between treatments. Our results suggest that allocating resources to maximise fitness during one life-stage, does not necessarily translate into higher fitness in the following life-stage.

ORAL 15:40-16:00

Transcriptomics of olfactory communication mediated by sex pheromones in *Bicyclus anynana*

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Olfactory communication is fundamental to most living organisms. In particular, sex pheromones are used for intraspecific communication between potential mating partners. Sex pheromones were recently shown to play a role in mate choice in the model butterfly *Bicyclus anynana* (1-4) and in reproductive isolation across the *Bicyclus* genus (5). Here we build on knowledge on the molecular bases of olfaction acquired for different insects, and investigate the whole sequence of steps and relevant body structures involved in sex pheromone communication in *B. anynana*. We have sequenced the transcriptome associated to i) olfactory perception (using antennal tissues), ii) sex pheromone biosynthesis (adult wings), iii) regulation of sex pheromone biosynthesis (brains), as well as iv) development of androconia, the sex pheromone-producing wing structures (pupal wings). High-throughput sequencing of eight distinct libraries produced a dataset of ˜700,000 reads, which we assembled (˜120,000 contigs) and annotated. With this, we could not only identify which genes are expressed in association with different steps and tissues, but also compare levels of expression between sexes and/or tissue sections. We followed this up with a more detailed characterization of the expression (qPCR) and phylogenetic analysis (comparison to data available for *Drosophila*, moths and *Heliconius* and *Danaus* butterflies) of selected candidate genes involved in sex pheromone synthesis (by males) and reception (by females). This is the first integrative molecular analysis of all functions associated to sex pheromone communication in a lepidopteran, which we aim to relate to the available behavioural, electro-physiological, and life history data on sex pheromone communication in this species. References: 1. Costanzo and Monteiro (2007) Proc Roy Soc B 274:845; 2. Nieberding et al (2008) PLoS ONE 3:e2751; 3.Nieberding et al (2012) Ecology Letters 15:415; 4. van Bergen et al (2013) Proc Roy Soc B 280:1471; 5. Bacquet et al (in revision)

ORAL 16:00-16:20

Genetic variation in diapause-related genes in *Pararge aegeria*

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Environmental variation is a significant cause of stress for organisms, which can vary spatially across habitats or temporally across seasons. In insects, one widespread adaptation for mitigating stressful periods is to enter a state of arrested development called diapause. The decision of whether to go into diapause is generally triggered by seasonal cues such as photoperiod. The diapause decision has a genetic basis and it often shows local adaptation along latitudinal clines. This is apparent in the butterfly *Pararge aegeria* where there is such a difference between populations that at 18 hours of light 100% of individuals of populations from northern Sweden will go into diapause while 100% of populations in southern Sweden will develop directly, when held under identical lab conditions. However, the genetic mechanism that underlies this local adaptation is unknown. At the moment we are searching for genetic differences in candidate genes by comparing population re-sequencing data from both a northern and a southern population of Speckled Wood. First results look promising.

ORAL 16:20-16:40

Multiple origins of industrial melanism in the peppered moth

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Industrial melanism in the peppered moth (*Biston betularia*) is a classic example of rapid adaptation to environmental change. Our studies of the underlying genetics have revealed different mutational origins of this polymorphism in the UK, continental Europe and North America. These different histories have implications for the way in which this adaptation has spread and the maintenance of the genetic polymorphisms and linked genetic diversity. The repeated involvement of the same gene in wing pattern adaptation in this and other Lepidoptera indicates a conserved developmental master switch, segments of which are highly labile.

ORAL 17:00-17:20

Effects of predictable and unpredictable juvenile conditions on adult life history: an RNA seq analysis in a butterfly

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Phenotypic plasticity is the ability of a given genotype to express different phenotypes in response to environmental variation. Many instances of phenotypic plasticity are adaptive, allowing organisms to cope with fluctuations in habitat quality, for example in seasonal habitats. Understanding how a single genome can express distinct, alternative phenotypes is crucial for a better mechanistic understanding of this important and widespread adaptation. Moreover, studying the regulatory basis of plasticity also provides more general insights into how genes and the environment interact to produce complex phenotypes. We use the butterfly *Bicyclus anynana* to study the transcriptomic basis of phenotypic plasticity. This tropical insect has evolved phenotypic plasticity of adult life history strategy as adaptation to its savannah habitat's contrasting seasonal environments. Adults of the affluent wet season reproduce fast and live relatively short lives, while those of the harsh dry season delay reproduction and live longer. These seasonal adult life history strategies are partly determined by environmental conditions during development, with low and high larval temperatures inducing the dry and wet season form, respectively. In addition, unpredictable food deprivation during development also affects adult life history, often in a season-specific manner. Here, we examine the genetic and transcriptional regulation of responses to both predictable (seasonal) and unpredictable (food stress) juvenile environments. We combine these two different environmental manipulations in a full-sib quantitative genetics design with sequencing of the mRNA pool (RNAseq). This yields transcriptome-wide estimates of gene expression variation as a function of genetic background (G), juvenile environment (E) and their interaction (GxE). The latter indicates genetic variation for plasticity at the transcriptome level. Furthermore, we use phenotypic measurements within the same experiment to correlate expression variation with plastic traits (metabolic rate, reproductive allocation, mass). Our study provides new insights into the regulatory links between juvenile conditions and adult life history.

ORAL 17:20-17:40

The pheromone biosynthesis activating neuropeptide (PBAN): a conserved molecular mechanism for regulating sex pheromone production across Lepidoptera?

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While sex pheromone biosynthesis has been studied extensively in moths, the corresponding molecular mechanisms remain largely unknown in butterflies. Here we combined behavioural, chemical and molecular approaches to assess whether the Pheromone Biosynthesis Activating Neuropeptide (PBAN), which controls sex pheromone production in moths, is also used in the model butterfly *Bicyclus anynana*. We build on previous results demonstrating that male sex pheromone (MSP) plays a key role in mate choice. First, we showed that there is daily variation in courtship activity and males are most active between 8 and 12 hours after sunrise. Second, using a family-design, we showed that MSP production varies throughout day on a 24- hour period such that males have maximum amount of hexadecanal (MSP2) when courtships occur most frequently. Third, using the genomic and transcriptomic datasets available for *B. anynana*, we identified a candidate gene sequence for PBAN in *B. anynana* and showed, using quantitative real time PCR, that PBAN gene activity exhibits over a 24 hour-period a daily variation in transcript abundance. The temporal variation of PBAN gene expression correlates to variations in both courtship activity and MSP production. Results together suggest that a key molecular mechanism regulating pheromone biosynthesis in moths may have been conserved across Lepidoptera for over 100 millions of years.

ORAL 17:40-18:00

Evolution of dominance in a polymorphic butterfly involved in Müllerian mimicry

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Butterfly wing colour patterns have provided robust evidence of positive selection due to the straightforward prediction of the direction of selection, such as in the example of melanism in the peppered moth. Within species polymorphism in wing patterns also provides a powerful model to study the effect of balancing selection on complex traits. In the case of mimicry among toxic species, positive frequency dependent selection often leads to the fixation of a single morph within population. However, in some rare cases, polymorphism of wing pattern can be maintained locally, presumably because of an equilibrium between local mimicry selection and migration at fine scale. Here we focused on the unpalatable butterfly *Heliconius numata*, which maintains several wing patterns within localities, each displaying high resemblance with other distasteful species from the local communities. In *H. numata*, wing pattern variations are controlled by a single locus, the supergene P, which shows several inversions in gene order among alleles, preventing recombination and effectively locking together the mimetic combinations. However, given the high polymorphism observed in natural populations, selection exerted by mimicry also acts on the phenotype of heterozygotes and therefore on the dominance relationships between mimetic alleles. The possibility of predicting the direction of selection by looking at the local mimetic communities represents a rare opportunity to investigate how balancing selection influences the evolution of dominance. We thus built theoretical simulations testing the invasion of dominance modifiers in such system, assuming enhancers and repressors cis- or trans- acting on the expression of alleles at the supergene P. We investigated the impact of these mechanisms of action together with recombination rates on the fixation probability of modifiers. We then compared our predictions with the variations of dominance observed in natural populations of *H. numata* to understand the evolutionary mechanisms shaping dominance in polymorphic adaptive loci.

TUESDAY 12.8.2014

MORNING SESSION: Genomics and Development

PLENARY 09:00-10:00

The origin and evolution of novel gene regulatory networks

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How do novel complex traits originate at the developmental level? And how do these traits become sensitive to the environment to evolve adaptive plasticity? I will report recent work that suggests that novel traits may originate *via* the recruitment of pre-existing gene regulatory networks. In particular, nymphalid eyespots appear to have evolved from simple colored spots concurrently with the recruitment of several genes to the eyespot centers. In addition, eyespots originated as individuated units on the ventral hindwing, which later colonized additional wings and wing surfaces. Ongoing work aims to identify the primitive gene network

that was recruited to make eyespots using transgenetic tools and reporter constructs containing candidate bits of regulatory DNA. In some lineages, such as *Bicyclus anynana*, the eyespot gene network evolved sensitivity to rearing temperature in an adaptive way. Eyespot plasticity evolved *via* modifications to the eyespot gene network and potentially to other networks involved in hormonal synthesis and regulation. We are currently investigating how each of the components necessary for the origin of plasticity evolved and came together in a large tree of nymphalids.

ORAL 10:30-10:50

The origins of dennis: Evolutionary innovation through modular regulation of an input-output gene

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The orange dennis-ray pattern is an evolutionary novelty that dominates Amazonian butterfly mimicry patterns. Up to 11 *Heliconius* species and both pierine butterflies and pericopine moths share this pattern, making this one of the most diverse mimicry rings known. Here, we have shown that this novel phenotype has arisen in *Heliconius melpomene* by combining two distinct elements that arose in divergent lineages on the *Heliconius* phylogeny. It has been hypothesised that cis-regulatory evolution might commonly occur through movement of CREs around the genome *via* transposition, and our data imply that adaptive introgression can similarly transfer CREs, and in the process generate novel phenotypic combinations. We also identify an upstream patterning gene that likely controls the expression of optix, and shows a conserved pattern of expression across the insects. Our results provide strong support for the input-output gene hypothesis and demonstrate the striking modularity of CREs even among recently derived expression patterns.

ORAL 10:50-11:10

New genes and new microRNAs in lepidopteran evolution

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To investigate how developmental patterning has changed during the evolution of Lepidoptera, we have obtained genome sequence data for five species from divergent evolutionary lineages within Lepidoptera plus a caddisfly outgroup, together with selected transcriptome and small RNA library datasets. We find evidence for new genes and new mRNAs arising at the base of the Ditrysia clade and suggest how they were recruited into the developmental programmes of Lepidoptera.

ORAL 11:10-11:30

Doublesex is a mimicry supergene

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One of nature's most striking examples of sexual dimorphism is sex-limited mimicry in butterflies, a phenomenon in which one sex, usually the female, mimics a toxic model species while the other sex displays a different wing pattern. Sex-limited mimicry is phylogenetically widespread in the swallowtail butterfly genus *Papilio*, where it is often associated with female mimetic polymorphism. In multiple polymorphic species, the entire wing pattern phenotype is controlled by a single Mendelian "supergene". While theoretical work has explored the evolutionary dynamics of supergene mimicry, there are almost no empirical data that address the critical issue of what a mimicry supergene actually is at a functional level. Using an integrative approach combining genetic and association mapping, transcriptome and genome sequencing, and gene expression analyses, we have found that a single gene, doublesex, controls supergene mimicry in *Papilio polytes*. This is in contrast to the long-held view that supergenes are likely to be controlled by a tightly-linked cluster of loci. Analysis of gene expression and DNA sequence variation suggest that isoform expression differences contribute to the functional differences between dsx mimicry alleles, and protein sequence evolution may play a role as well. Our results combine elements from different hypotheses for the identity of supergenes, showing that a single gene can switch the entire wing pattern among mimicry phenotypes but may require multiple, tightly-linked mutations to do so.

ORAL 11:30-11:50

The Glanville fritillary genome retains an ancient karyotype and reveals exceptional chromosome stability in Lepidoptera

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Previous studies on lepidopteran genomes and karyotypes have reported two seemingly contradictory results: chromosome macro-synteny is high, yet the number of haploid chromosomes varies widely from 5 to 223. The ancestral chromosome number in Lepidoptera has been conjectured to be the modal n=31, but it has not been possible to test this hypothesis. The Glanville fritillary butterfly (*Melitaea cinxia*), for which the genome and high-density linkage map are now available, has the putative ancestral karyotype of n=31. Our comparative analysis of the genomes of the Glanville fritillary and other lepidopteran species demonstrates that high macro-synteny is due to conservation of the ancestral chromosomal contents. Using a high-resolution phylogenetic analyses of Nymphalidae (312 species) and a broader analysis of other Lepidoptera, combined with ortholog-level comparisons of chromosomes, we conclude that the ancestral lepidopteran karyotype has been n=31 for at least 140 My. We show that fusion chromosomes have retained the ancestral chromosome segments and very few subsequent rearrangements have occurred across the fusion sites. However, intrachromosomal rearrangements do occur. The same, shortest ancestral chromosomes have independently participated in fusion events in species with smaller karyotypes. The short chromosomes have more transposable elements and exhibit higher evolutionary rate than long ones. Our results show exceptionally high chromosome stability in Lepidoptera in terms of macro-synteny and constrained fusions, but conversely relatively high evolutionary rate within chromosomes. These characteristics highlight distinctive features of the evolutionary dynamics of butterfly and moth genomes, which appear to evolve in a different manner than most other metazoan genomes.

ORAL 11:50-12:10

Genomics and the evolution of central metabolism in butterflies

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Understanding the tempo and mode of gene evolution is a central goal of evolutionary biology, yet even for central metabolism we lack a coherent framework for understanding the large differences in polymorphism, divergence, and codon bias among these genes. Emerging from studies on model systems is a view that the pathway location and the regulatory role of a given enzyme affects that enzyme's molecular evolutionary dynamics. Here I present my recent attempts to explore this nascent systems view using genomic data from butterflies and other insects.

AFTERNOON SESSION: Butterfly movement

KEYNOTE 13:30-14:10

Butterfly movement: when to go and when to stay

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Butterfly movement is influenced by phenotype, habitat and abiotic environmental conditions, at a minimum. Much previous work has looked at movement in the context of habitat edges, land use characteristics and metapopulations, including genetic and physiological mechanisms underlying differential activity levels. I first review and synthesize highlights from the literature. I then address work by myself and members of my lab, primarily on *Speyeria mormonia*, examining factors affecting variance among years in dispersal within a heterogenous grassland habitat.

ORAL 14:10-14:30

Species traits as filters of climate-induced range expansion

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As climate warms, species are expected to move poleward or up in elevation to track the environmental conditions to which they are most adapted. However, this movement and the subsequent colonization of new habitat may be affected by species-specific habitat affinities and life history traits that facilitate or impede range expansion. Even if the "thermal envelope" is ideal, the vegetation conditions or other environmental parameters may not be conducive. Similarly, traits such as wingspan or voltinism may act as a filter in defining which species can successfully colonize new regions. We used 100 well-sampled butterfly species to track changes over time in Canada, comparing data from 1880–1970 and 1985–2012. The country was divided into a set of grid cells at a 4-degree resolution and species distribution changes were assessed within each grid cell. Because there were differences in the total number of species observed between the two time periods, we used a standardized difference measure to assess changes in relative abundance for each species. We modeled the standardized difference as a function of latitude and species traits, including wingspan, habitat preference (open, closed, or edge), moisture affinity (xeric, mesic, or both), and voltinism. Species with larger wingspans and larger numbers of generations per year showed positive trends in abundance. Species associated with open habitats or edges, and species that could be found in multiple habitat types (both xeric and mesic) also showed increasing trends. We examined whether

the latitude where a species had been observed increased between these two time periods and modeled the probability that a species had moved north as a function of these life history traits. Species associated with xeric habitats had ˜8 times higher odds than those associated with mesic habitat to have moved northward between the two time periods.

ORAL 14:30-14:50

Does habitat use reflect habitat quality? A comparison of three butterfly species in restored and remnant habitat patches

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Habitat use is often used as a metric of habitat quality, especially in wildlife conservation. However, this assumption is rarely explicitly tested. Notable exceptions such as ecological traps are familiar, but we do not know if these are rare cases, or if habitat use is generally a poor metric of habitat quality. We explored these relationships for three butterfly taxa (the Fender's blue (*Plebejus icarioides fenderi*), the Baltimore checkerspot (*Euphydryas phaeton*), and the Appalachian brown (*Satyrodes appalachia*)) across a range of remnant and restored habitat patches. Restoration techniques included burning to remove shrubs from upland prairies, and damming/hardwood removal to create wet meadows. Habitat quality was quantified by measuring vital rates (birth, survival), and calculating habitat-specific population growth rates. Habitat use was quantified by mapping flight paths of individual butterflies, and using (biased) correlated random walk models to calculate habitat-specific diffusion coefficients. Within each taxon, habitat use reflected habitat quality. Butterflies had lower diffusion coefficients and would therefore be expected to spend more time in patches for which they had higher intrinsic growth rates. This result implies that habitat use is generally useful as a coarse metric of habitat quality, even in novel habitat types. I also discuss the merits and constraints of measuring habitat-specific population growth rates and diffusion coefficients directly in the field, as metrics of long-term demographic and movement dynamics.

ORAL 15:20-15:40

Connectivity and the genetic effects of a population collapse

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Dispersal and connectivity mediate gene flow, and therefore play a key role in determining levels of genetic diversity within populations, and patterns of spatial genetic structure among populations. Population size fluctuations also influence population genetics. While independent effects of changes in population size and connectivity on genetic patterns and processes are well studied, a key challenge is to understand their interaction. This is increasingly important as natural populations are being simultaneously exposed to both habitat fragmentation, which reduces connectivity, and climatic changes, which increase variability in population size. Using a combination of temporally replicated demographic, dispersal, and genetic data, we examined the interaction between connectivity and a severe demographic collapse on population genetic structure in a network of populations of the alpine butterfly, *Parnassius smintheus*. In this population network, abundance declined 60–100% in 2003 because of low over-winter survival. A comparison of population genetic diversity and structure before and after the collapse, based on microsatellite genotyping, indicated no change in genetic diversity across the network. However, patch connectivity and local severity of the collapse (population size within a patch during the year of the collapse) interacted to determine allelic richness change within populations. This result suggests that dispersal and connectivity within a population network can mediate the genetic response to a demographic collapse, by contributing to genetic rescue via immigration. The collapse was also associated with a dramatic change in spatial genetic structure, and lead to a two-fold increase in differentiation, breakdown of isolation by distance, and loss of landscape genetic patterns. Our ability to combine long-term demographic, dispersal and genetic data for a population network has revealed important interactions between connectivity and temporal demographic variability on the genetics of populations.

ORAL 15:40-16:00

Flight and dispersal in the Glanville fritillary butterfly: genes, physiology and environmental effects

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Insect flight is energetically one of the most demanding forms of locomotion in the animal kingdom. Nonetheless, flight is critical for foraging, reproduction and dispersal in most insects, especially those inhabiting fragmented habitats.

We have investigated the genetics and physiology of flight in the Glanville fritillary butterfly (*Melitaea cinxia*), a well-established ecological model species for studying the consequences of habitat loss and fragmentation. Previous studies have shown that the rate of metabolism during flight predicts well the dispersal rate of individual butterflies in the field. We show that flight metabolic rate exhibits significant heritability, indicating responsiveness to changing selection pressures on dispersal. To understand in greater detail the genetic mechanisms affecting flight, we studied changes in gene expression induced by active flight. We found 755 genes which responded to flight by significant changes in expression. The majority of these genes were related to metabolic processes and responses to stress and hypoxia. There were significant differences in flight-induced gene expression between females and males as well as between butterflies originating from populations with dissimilar genetic backgrounds. These differences in gene expression were associated with differences in flight metabolism and hence dispersal capacity. Further, flight metabolism interacts with prevailing environmental conditions, especially ambient temperature, in affecting realized flight behavior. The aim of our research is to build a better understanding of how genes, physiology and the environment play together in influencing dispersal in the Glanville fritillary and other butterflies.

ORAL 16:00-16:20

The population dispersal and conservation needs of montane endemic butterflies in Southern Mexico

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Mexico has a huge number of endemic insect species; some of these species are associated with relict habitats and small surviving populations, and thus are conservation targets. Tropical montane endemic species could be more susceptible to anthropocentric effects –including global warming – compared to relatively widespread species. We assessed the role of dispersal ability and ecological requirements upon the persistence of key montane butterfly endemics in southern Mexico. Our results emphasize the need to perform ecological assessments to direct prevention or restoration actions to mitigate anthropogenic impacts and promote tropical insect conservation.

ORAL 16:20-16:40

Movement-based approaches for estimating population growth rates and critical minimum patch size

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Determination of the minimum patch area required to sustain a population has a long history in theoretical and conservation biology. Based on the principles of diffusion, the critical minimum patch size can be defined as the smallest area within which individuals of a population remain long enough to reproduce and replace themselves in future generations. Population viability is often assessed via the minimum population size required for stable population growth in the presence of environmental, genetic and demographic stochasticity. In contrast, the idea of critical minimum patch size ignores other forms of stochasticity, largely focusing on the fact that, in populations with random movement, individuals in smaller patches will encounter edges and leave with higher probability than in larger patches, and potentially before reproducing within the patch. We determined the critical minimum patch size for the Baltimore checkerspot butterfly, *Euphydryas phaeton*. *E. phaeton* is locally abundant but declining along its southern range limit, making estimates of minimum patch area of conservation value. We used two related approaches: an analytical model built from theoretical principles and field-collected movement data, and an individual-based simulation using movement and demographic data. We estimate residence times and population growth rates from both models, and show the range of patch sizes across which model parameters are in close agreement. We introduce individual variation and sequentially remove demographic information from simulated models, and report robustness to initial comparison with analytical models. We expect that our analytical approach can be applied to other species whose movement follows the assumptions of diffusion and where demographic data are limited. We compare our conclusions for minimum patch size based on movement to alternative methods for assessing minimum area requirements (e.g., based on life history characteristics) for butterfly populations.

ORAL 17:00-17:20

Higher mobility of butterflies than moths connected to habitat suitability and body size in a release experiment

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Mobility is a key factor determining lepidopteran species responses to habitat changes. However, direct multi-species comparisons of mobility are rare and empirical comparisons between butterflies and moth groups have not been previously conducted. Here, we compared mobility between butterflies and diurnal moths and studied species traits affecting butterfly mobility. We experimentally marked and released 2011 butterfly and 2367 moth individuals belonging to 32 and 28 species, respectively, in a 25 m 25 m release area within an 11-ha, 8-year-old set-aside field. Distance moved and emigration rate from release habitat were recorded by species. The release experiment was successful in producing directly comparable mobility data in 18 butterfly and 8 moth species with almost 500 recaptured individuals. Butterflies were found much more mobile than geometroid moths both in terms of distance moved (mean 338 m vs. 63 m, respectively) and emigration rate (mean 56% vs. 17%, respectively). Mobility of noctuoid moths was somewhere between butterflies and geometroids. Release habitat suitability had a strong effect on emigration rate and distance moved, because butterflies tended to leave the set-aside field if it was not suitable for breeding. Emigration rate and distance moved also increased significantly with increasing body size. When phylogenetic relatedness among species was included in the analyses, the significant effect of body size disappeared for distance moved, but habitat suitability remained significant. The much higher mobility of butterflies than geometroid moths can largely be explained by differences in robustness of their flight morphology. The important role of release habitat suitability for butterfly mobility was expected, but seems not to have been empirically documented before. The observed positive correlation between butterfly size and mobility is in agreement with our previous findings on butterfly colonization speed in a long-term set-aside experiment and recent meta-analyses on butterfly mobility.

ORAL 17:20-17:40

Multicausality of dispersal variation in a butterfly metapopulation

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Climate change and habitat loss are among the main drivers behind the current biodiversity crisis, but there is still limited understanding of the detailed mechanisms behind these patterns. Building predictive models of the consequences of global change at the level of populations and species is especially challenged by the difficulty of understanding how animals move through fragmented landscapes, and how the movements are affected by the changing environmental conditions. We tackled this challenge in the context of one of the rare long-term data sets on insect movement, comprising >20 years (1992–2012) of capture-recapture data on the threatened bog fritillary butterfly (*Boloria eunomia*) inhabiting a fragmented landscape in Belgium. We used a spatially explicit state-space modelling approach with Bayesian inference to estimate key dispersal parameters, including habitat selection (preferences for high quality habitat, low quality habitat, and the unsuitable matrix), habitat- specific movement rates, and mortality. Movements varied substantially among the years, and co-varied systematically between the two sexes. Our results illustrate that movements are affected by a complex interaction between internal and external factors, suggesting that it is difficult to predict the impacts of climate change on insect movements even in well-studied model systems such as the bog fritillary.

ORAL 17:40-18:00

Importance of dispersal and metapopulation dynamics in tropical butterfly conservation

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Majority of butterfly biologists and conservationists find metapopulation theory important for butterfly conservation. Indeed there are many in-depth studies of butterfly dispersal, movement and metapopulation dynamics which have directly contributed to temperate butterfly conservation e.g., in guiding landscape management of endangered Fender's Blue butterfly and to identify conservation strategies for a rare South Carolina endemic butterfly *Atrytonopsis* new species 1. Yet there seems to be few metapopulation studies from the tropics, with relatively little known about the spatial structure and dispersal capabilities of most tropical butterflies. We reviewed 62 studies from tropical dispersal literature covering the Neotropics and Paleotropics and found a skewed representation of studies covering Nymphalidae followed by Papilionidae. Nymphalidae were represented by classic model research groups such as fruit-feeding nymphalids (Neotropics and Paleotropics), *Heliconius* and Ithomiine (Neotropics) and *Acraea* and *Bicyclus* (Afrotropics). Narrow range species, habitat specialists and rare and threatened species were all poorly represented. Thereafter, dispersal distances of tropical butterflies were extracted from a subset of 22 studies. They were scaled by wingspan and compared with dispersal distances of temperate butterflies (also scaled by wingspan). Dispersal distances of tropical butterflies are correlated with habitat quality, suggesting that dispersal distances of temperate butterflies can only be used with caveats for tropical butterflies. Our analysis further highlights the urgent need to quantify the effects of dispersal and movement in relation to habitat quality and fragmentation in the tropics and to incorporate them into tropical butterfly conservation and metapopulation dynamics research.

AFTERNOON SESSION: Sexual selection

KEYNOTE 13:30-14:10

Sexual selection, Darwin, and butterflies: Then and now

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Butterflies figured prominently in Darwin's development of sexual selection theory. This theory has proven to be a powerful and productive driver of research and valuable for understanding the evolution of many aspects of animal reproductive behavior. However, its relevance to our understanding of butterfly coloration and other aspects of their reproductive biology was point of contention in Darwin's time, and even today empirical evidence for how sexual selection in butterflies plays out in nature, as opposed to the lab, is scant. This talk will review some of the challenges and still unresolved issues that we face in evaluating the nature and importance of both pre- and post-copulatory sexual selection in butterflies. I will identify and motivate promising and important research questions such as how much variation in fitness in one sex can be explained by mate preferences in the other, the causes and consequences of sexual signal variation, whether spermatophores are mating or parental investment, the relative roles of visual and chemical signals in mate choice, how pre- and post-copulatory events interact, etc. I will highlight factors such as tracking individuals in the field that have limited progress in addressing these challenges. Continuing efforts to study sexual selection in butterflies should benefit from the diversity of behavior and ecology found among the 20,000 or so species, and should offer special opportunities to advance our understanding of how sexual selection acts on coloration and other aspects of sexual behavior in animals generally.

ORAL 14:10-14:30

Sexual coevolution and Lepidoptera genitalia

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In Lepidoptera, in general, male and female genitalia are complex structures that evolve rapidly and divergently. This pattern is shared with many other animal groups and sexual selection is considered the main reason for this phenomenon, although the importance of different types of sexual selection has been debated. I will give a glimpse of the enormous diversity and exuberance of Lepidoptera genitalia, and I will argue that their study could shed light on questions that have been difficult to answer in other groups. In particular, I will discuss the idea that the female's corpus bursae is a "playing field" for sexual coevolution of genital traits. During and after copulation, male-female interactions take place in the corpus bursae that affect the reproductive success of both sexes and could result in the evolution of reciprocal adaptations. I will illustrate these points with studies on: (1) the coevolution between spermatophore envelopes and the signa located in the corpus bursae, and (2) the diversity and possible functions of the deciduous genital spines located in the endophallus of several species. (Financial support: PAPIIT-UNAM IN208413.)

ORAL 14:30-14:50

Butterfly mate preference learning: Sexually dimorphic learning, learning biases, and multimodal signaling

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Mate preference learning occurs in many animal taxa, with and without parental care. In species without parental care, such as butterflies, it is unclear whether, and how, animals discriminate between potential preference models in their environment and form non-random mate preferences for adult phenotypes. Using the butterfly *Bicyclus anynana* we conducted a series of mate choice and learning assays and tested two possible mechanisms of discrimination: learning biases and facilitative signals in other sensory modalities. We found that *B. anynana* butterflies exhibit mate preference learning biases. Naïve female *B. anynana* butterflies prefer males with wild-type forewing ornamentation (two dorsal eyespots) to males with more forewing ornamentation (extra dorsal eyespots), and learn preferences for more, but not less, forewing ornamentation. Male *B. anynana* butterflies also exhibit mate preference learning biases, but they learn preferences for a different trait, and their learning is biased in a different direction from female forewing preference learning. Males learn preferences for females with less, but not more, hindwing ornamentation. In addition to exhibiting visual learning biases, female preference learning is mediated by olfactory signals. Females exposed to males with manipulated odor do not learn to prefer extra ornamentation, and learn to avoid the wild-type male ornamentation. We therefore demonstrate that *B. anynana* butterflies have at least two different mechanisms that facilitate discrimination between potential visual preference models: learning biases and olfactory signals. Furthermore, we demonstrate that mate preference learning in *B. anynana* is sexually dimorphic in terms of what traits are learned and direction of learning biases. These findings suggest that multimodal signaling may facilitate model discrimination, and illustrate the potential for sexual dimorphism in learning ability to influence the evolution of sexually dimorphic ornamentation.

ORAL 15:20-15:40

The interplay between different stages of reproduction in *Plodia interpunctella*

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Traditionally studies of sexual selection have focused on individual 'episodes' of reproduction in isolation. However, an individual's reproduction often comprises a number of successive stages, which can work in concert to affect overall reproductive fitness, or can interact antagonistically with one another. Thus, to fully understand the total sexual selection operating on an individual, all aspects of reproduction, across total lifespan, have to be examined. We measured lifetime reproductive success, both pre-copulatory and post-copulatory, in male Indian meal moths (*Plodia interpunctella*). We found that males that were better at seducing non-virgin females were also better in obtaining matings overall. Male ability to transfer sperm was linked to sperm competition ability, and ability to reduce female remating, suggesting that sperm production and transfer are key determinants of overall reproductive success in this species. We found no indication of a link between traits contributing to both pre- versus post-copulatory reproduction. Our results highlight the importance of examining traits that affect both pre- and post-copulatory reproductive success, in order to understand the total sexual selection operating on males.

ORAL 15:40-16:00

Proteomic analysis of eupyrene and apyrene sperm in Monarch butterflies

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Why would males make sperm – lots of them – that have no DNA and don't fertilize eggs? This unintuitive phenomenon is commonplace among Lepidoptera, where most species exhibit sperm heteromorphism. Lepidopteran males typically produce two kinds of sperm: eupyrene sperm contain a nucleus and DNA, while apyrene sperm lack these features and are presumably incapable of fertilization. The functional and evolutionary significance of apyrene sperm remains poorly understood. Artificial insemination experiments in silkworm reveal that removing apyrene sperm dramatically reduces fertilization rates, demonstrating that apyrene sperm have a fundamental role in reproduction (Sahara & Takemura, 2003, J Exp Zoo). Yet apyrene sperm are also implicated in delaying female remating in *Pieris* butterflies, demonstrating an important role in postcopulatory sexual selection (Cook & Wedell, 1999, Nature). Here we report the results of applying "shotgun proteomic" experiments to comprehensively characterize the proteomes of apyrene and eupyrene sperm in Monarch butterflies (*Danuas plexippus*). First we developed a novel and straightforward method of co-isolating the two sperm morphs from males that reliably provides high yields of both sperm types for subsequent analysis. Subsequent LC-MS/MS analysis of the apyrene and eupyrene sperm fractions identified >1000 proteins in each proteome. Detailed investigations of these results are ongoing and will form the foundation for functional and evolutionary genomic analysis of the differences identified between apyrene and eupyrene sperm in Monarch butterflies.

ORAL 16:00-16:20

Coexisting closely related species under the same disguise: insights into species recognition issues between *Heliconius hecale* and *H. ismenius*

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In Lepidoptera, the factors promoting diversification and speciation include both coloration and semiochemical communication, implicated in inter and intraspecific communication as well as in sexual selection. Toxic butterflies in the neotropical genus *Heliconius* exhibit a spectacular diversity of wing patterns, which are aposematic. Furthermore, multiple unpalatable species often converge to a common wing pattern through Müllerian mimicry. Since color is involved in mate choice, confusion between co-mimics can happen in nature. However, distantly related co-mimics may use multiple mating signals, presumably reducing the confusion due to wing-pattern similarity. *Heliconius ismenius* and *H. hecale* are closely related species. They coexist in Eastern Panama and are excellent co-mimics of each other (*H. i. boulleti*and *H. h. melicerta*). In contrast, in Western Panama they display distinct patterns, belonging to distinct mimicry rings (*H. i. clarescens* and *H. h. zuleika*). We explore whether the species boundaries are more permeable to gene flow when the two species are co-mimics. We hypothesize that coloration similarities would favor hybridization between *melicerta* and *boulleti*, both at the pre-zygotic level, as expected if assortative mating based on color is weak, and at the post-zygotic level, if hybrids look very similar to parental taxa. Alternatively, other short-range signals such as pheromones might have evolved as barriers to hybridization. To understand the respective role of color and semiochemical communication in hybridization, we carried out a genome-wide analysis of gene flow across Illumina-resequenced genomes of the four taxa, as well as mating experiments involving *boulleti* and *melicerta*, and analysis of the chemical cocktails extracted from different tissues. Our results suggest that chemical signals might be important for species recognition, especially for female choice. Concurrently, gene flow appears to be stronger between the mimetic pairs, which could be partially operated by male choice based on color. Our data bring new light to our knowledge of the forces driving diversification in butterflies.

ORAL 16:20-16:40

Male ejaculates and female reproductive adaptations in the polyandrous butterfly *Pieris rapae*

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In most internally fertilizing animals, males transfer their gametes along with a diverse set of ejaculate compounds. The function and evolutionary significance of these ejaculate constituents has become an active area of research. However, this growing literature has focused largely on the male perspective, leaving many questions open regarding how female traits mediate the costs and benefits of male ejaculate substances. For instance, researchers have struggled to identify ways in which females gain information about male ejaculate characteristics prior to mating. In addition, research on the functional significance of male ejaculate compounds has typically evaluated higher-order female responses such as lifespan, fecundity and remating rate. As a result, we still know little about specific female adaptations responsible for mediating the influence of male ejaculate compounds. We report the results from an ongoing series of studies in the Cabbage White butterfly, *Pieris rapae*, that seek to address these knowledge gaps. First, we show that female *P. rapae* selectively mate with larger, more colorful males, and that these males transfer larger ejaculate packages, called spermatophores, during mating. Thus, specific male traits appear to provide females with information about spermatophore quality prior to mating. However, while these large male spermatophores benefit females by increasing lifespan and reproductive output, they also provide males with a means of constraining female remating rate because females do not mate again until they have sufficiently digested the male spermatophore. We discuss our ongoing efforts to characterize the evolutionary history and current function of specific female traits involved in spermatophore digestion within the female reproductive tract, including muscular and morphological traits that provide mechanical digestion as well as molecular traits engaged in chemical digestion of spermatophore substrates. We discuss the potential that these traits may have evolved as adaptations in the context of sexual conflict over female remating rate.

ORAL 17:00-17:20

Sexual conflict and the evolution of novel warning patterns in a Müllerian mimic

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Spectacular mimicry in *Heliconius* butterflies is a textbook example of evolution by natural selection. Because predators learn to associate particular patterns with unpleasant experiences, an individual's risk of predation decreases as the local density of its warning pattern increases. However, *Heliconius* are also known for their diversity of warning patterns, and the establishment of entirely new phenotypes is paradoxical under strict number-dependent selection. One possibility is that during periods of relaxed selection, drift may allow new variants to rise above a threshold density until mimicry selection takes over. I propose an alternative hypothesis where novel pattern phenotypes arise due to a conflict of interests between the sexes. It is established that male *Heliconius* use warning patterns as a mating cue. This is likely beneficial to males as it will increase the efficiency of finding mates. However, already mated females may suffer fitness costs if these cues lead to harassment by males during oviposition or foraging. When constraints imposed by predation are locally relaxed, this may lead to rapid divergence in pattern phenotypes through chase-away sexual selection: novel patterns allow females to resist fitness costs imposed by males, but this is countered by the evolution of corresponding male preferences. To begin to explore this hypothesis, I present a spatial individual-based simulation model, based on the biology and genetics of a *Heliconius* butterfly. I use this model to consider scenarios under which male harassment of females initiated by pattern recognition results in the spread of novel pattern alleles. This is compared to simulations where the effects of drift are expected to be the most pronounced. Finally, I discuss planned empirical work to test the key assumptions of this hypothesis. To my knowledge, sexual conflict has not previously been considered as a driver of pattern divergence in a Müllerian mimic.

ORAL 17:20-17:40

Evolutionary patterns of male sexual characters across Mycalesina butterflies

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The Mycalesina butterflies comprise a subtribe within the Nymphalidae family, with around 300 recognised species. Many species are morphologically very similar, but the males show a wide range of sexual characters of which one in particular, the androconial structures, has formed the basis of the taxonomy of the six genera within the subtribe. The speciose genus *Bicyclus* is found all over sub-Saharan Africa. *Heteropsis* is the most widespread genus – some species co-occur with *Bicyclus* on the African mainland while most of the Afro-tropical species are part of a radiation endemic to Madagascar, and there are also a few Asian representatives. *Mycalesis* and *Mydosama* occur in South-East Asia and the Australasian region. The males of *Bicyclus* are known to produce large quantities of sex pheromones used at close range during courtship. Recently, similar pheromone systems have been documented in *Heteropsis*, *Mycalesis* and *Mysodama*. Comparing the genera it appears as if *Bicyclus* have the most complex androconial system (structures that to some degree are linked with the chemical communication), while the other genera have a much more complex patterning on the dorsal wing surface, a trait shown to be involved in mate choice. The male genitalia structures among species are also variable, but here the variation appears to be more complex with no clear pattern to date. Initial comparisons of pheromones suggest that the complexity is much less pronounced in *Heteropsis*, especially the Madagascar species, that instead show a wider diversity of wing patterns. Since all these traits are likely to be involved in sexual selection we argue that systematically measuring these traits and mapping them onto a multi-gene molecular phylogeny and examining for correlative patterns will help to understand the evolution of mating and species recognition systems.

ORAL 17:40-18:00

Testing for cryptic female choice in monarch butterflies

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Sexual selection is an important force in shaping the evolution of species. Although many studies have focused on pre-copulatory female choice, it is increasingly clear that females may choose paternity following copulation with multiple males. Such cryptic female choice may be especially common in species where females have limited ability to choose pre-copulation. We tested for cryptic female choice in the monarch butterfly (*Danaus plexippus*). Whereas most butterfly species have elaborate pre-copulatory courtships, mating in monarchs is coerced by males, suggesting females only have post-copulatory opportunities to affect the fatherhood of their offspring. We used a mating design consisting of pairs of females, both mated to the same two males. Using microsatellite markers, we determined P2, the proportion of offspring fathered by the second male. In three treatments, we varied the relatedness of females and males, and calculated the repeatability of P2 values of the first and second female in a replicate. Assuming cryptic female choice, we predicted more repeatable P2 values for genetically related female pairs than unrelated pairs. In addition, we predicted that females should favor paternity by unrelated males over brothers to avoid potential inbreeding depression. Our results revealed no differences in repeatability of P2 values between treatments and no differences in paternity of brothers and unrelated males. These results suggest that monarch butterflies do not employ cryptic female choice and do not avoid inbreeding. Moreover, we did not find significant sperm precedence; neither the first nor second male obtained higher paternity. However, our results did indicate that interactions between male and female genotypes affected offspring paternity, suggesting that genetic compatibilities may affect sexual selection in this species. We also found a bimodal paternity distribution, confirming that monarchs follow the lepidopteran pattern of paternity, despite pre-copulatory behavioral differences.

WEDNESDAY 13.8.2014

MORNING SESSION: Ecology and Evolution

PLENARY 09:00-10:00

Diversification of the Lycaenidae: key characters involved in speeding up and slowing down

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Analysis of shifts in diversification rates in the butterfly family Lycaenidae reveals two lineages that have experienced dramatically increased rates of diversification, one within the Polyommatinae or “blues”, and another in the branch of the Theclinae that contains the hyperdiverse tribe Eumaeini. In contrast, a lineage in the carnivorous subfamily Miletinae has undergone a significant decrease in diversification. Research on the biology of taxa within these three lineages points to several key characters that may have influenced their rates of diversification.

ORAL 10:30-10:50

Evolution of host plant use in butterflies

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The associations between butterflies and their host plants have been the focus of intense study for half a century, but the evolutionary dynamics forming these associations are still not well understood. Most butterflies are specialized and very conservative over evolutionary time in their host plant utilization. With very few exceptions even the polyphagous species are better described as “polyspecialists” than as true generalists, in that they utilize a specific set of plant taxa that are also used as larval hosts by related butterflies. Yet host shifts do occasionally occur, sometimes to distantly related plant taxa, and they have been suggested to be associated with “escape-and-radiate”-type scenarios of diversification. Other studies rather point to host shifts being an almost incidental effect of a transient polyphagous and plastic evolutionary stage, as butterfly taxa oscillate from one specialized state to another. I give a general overview of these ideas and the current evidence for them from ecological, phylogenetic and genomic studies, and attempt to reconcile the two patterns into a coherent view of evolution of host use in butterflies.

ORAL 10:50-11:10

A tale of two communities: temporal and spatial turnover in vertically stratified tropical fruit-feeding nymphalid communities

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Neotropical butterfly communities are notable for their spectacular species richness. Fruit-feeding nymphalid butterflies are one guild that has received a great deal of attention and have been the subject of multiple long-term trap studies. Here, we examine community structure of these butterflies based on four long-term studies carried out in Central and South America. Although they occupy a similar feeding guild as adults, specializing on rotting fruit juices as adult food resources, the composition of canopy and understory communities has little overlap. There is a strong phylogenetic signal, with some lineages almost exclusively foraging in either canopy or understory habitats. Overall, very few species can be reliably found in both strata. Beyond the vertical partitioning of species occurrence, the dynamics of community composition varies greatly between canopy and understory. At each of the four study sites both spatial and temporal Beta-diversity, or species turnover, is much higher in the canopy compared to understory. On average, there were nearly 50% greater effective communities in the canopy compared to understory habitats at all study sites. We compared the community composition of intact rainforest with a comparatively more homogeneous abandoned banana plantation. Although richness and diversity was lower at the plantation site, the pattern of greater canopy species turnover compared to understory habitats remained. The difference between canopy and understory nymphalid communities extends beyond species composition, with each having a unique dynamic in time and space.

ORAL 11:10-11:30

Thoughts on the contributions of research on the biology of butterflies to the biosciences

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Having attended all of the ‘Biology of Butterflies’ meetings it seemed an idea to me to both look back, and step back a little, to consider the extent to which work that has made use of butterflies has led to advances and impact within the broader field of the biosciences. How has research on butterflies contributed to diverse areas of biology, and how have such contributions developed over the several decades since that first meeting in London? This short talk can only be a skim through such a wide field, and will of course be very much a personal choice that undoubtedly misses many breakthroughs; it will indeed be fascinating to hear the thoughts of other delegates. However, I believe such a survey, even if very incomplete, will highlight that butterfly biology punches way above its weight relative to the size of our community, and that in many respects this is a rich legacy of pioneering butterfly biologists, some of whom were present in London over 30 years ago.

ORAL 11:30-11:50

When canopy comes to the ground: effects of forest disturbance on community structure of tropical butterflies

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Fruit-feeding butterflies are considered one of the best groups for ecological monitoring, since they are relatively large, easy to sample and well-known taxonomically. In the last years, several studies contributed to a better understanding of the temporal and spatial patterns of distribution of butterfly assemblages in Neotropical forests. As a rule, richness-related results are not conclusive, but the species composition is constantly related to habitat structure, and the pattern of differences in composition between habitats can be observed also at higher taxon levels. Concerning the spatial dimension, recent results with vertical stratification of fruit-feeding butterflies in the Atlantic Forest and Amazonia in Brazil showed that Charaxinae are abundant in the canopy, while tribes Morphini and Brassolini (Satyrinae) are more common in understory. Interestingly, this pattern is similar to that observed in pristine vs. disturbed habitats, with Morphini and Brassolini more abundant in more conserved habitats, while Charaxinae proportionally increasing with disturbance. Forest disturbance has also been shown to strongly affect understory, but not the canopy. Comparative data from several Neotropical locations strongly suggests that forest disturbance affects the understory making it more similar to canopy (i.e. sunny, dry and warm), which should result in a gradual replacement of butterfly assemblages between these two strata. These results are of high importance to forest management and conservation in tropical habitats.

ORAL 11:50-12:10

Ecological drivers of sexual dimorphism and polymorphism in mimetic butterflies

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The hallmark of life on earth is morphological diversity, which is represented in the spectacular sexually dimorphic and polymorphic wing patterns of butterflies. Biologists have long tried to find general ecological explanations for the evolution of morphological diversity, especially at intraspecific levels. We address this problem by investigating the remarkable diversity of Batesian mimetic wing patterns in butterflies, manifested in numerous sex-limited or otherwise polymorphic forms within species. Historically, the major hypotheses to explain these polymorphic forms have centered around the concepts of sexual selection, physiological trade-offs, and shifting balance. While there is interesting evidence to support components of each of these ideas, a broad-ranging ecological explanation for this type of polymorphic wing patterns is still missing. Towards this end, we present a simple mathematical model that may explain the full gamut of polymorphic diversity exhibited by mimetic butterflies. Our model is based on frequency dependent natural selection (predation) and sex-specific advantages of mimicry, both of which are empirically established. Our model predicts that in ecological communities composed of toxic models and undefended mimics, different threshold mimic frequencies favor transitions between monomorphic, female-limited and polymoprhic mimicry. Data on 57 mimetic butterfly species showed that monomorphic mimicry was prevalent at low mimic frequencies whereas female-limited and polymorphic mimicry were prevalent at high mimic frequencies, as predicted by the model. We further show with controlled experiments on the *Papilio polytes* butterfly that female-limited mimetic polymorphism in this species could not be explained by sexual selection, physiological tradeoffs or other hypotheses. However, the presence and density of models appeared to determine the presence and frequency of mimetic forms in a given locality. Thus, frequency-dependent natural selection in the form of predation appears to be the predominant force driving wing pattern polymorphism in Batesian mimetic butterflies.

AFTERNOON SESSION: General butterfly biology

ORAL 13:30-13:50

What is the Mithcell's satyr butterfly? Contemporary approaches to an old question

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Butterflies of the genus *Neonympha* (Nymphalidae: Satyrini) are found in ecologically similar wetlands in the eastern United States. This genus has been recorded from states bordering the Gulf of Mexico to those along the Atlantic Ocean, and even from areas of North America that were covered by ice during the Pleistocene. Because of this broad distribution it should not be surprising that *Neonympha* has a complicated taxonomic history with species being lumped and split many times over. At present there are three species recognized in the genus but boundaries between these species are uncertain because much of the work that established them relied on small sample sizes and qualitative characters. The status of *Neonympha* taxa is important because two subspecies of *N. mitchellii* are classified by the federal government as endangered. Adding to the uncertainty has been the recent discovery of additional disjunct populations of *N. mitchellii*, which could have important implications for the legal status of the protected species. I applied contemporary statistical and genomic methods to delimit taxa and aid in the conservation of these rare butterflies. I first use geometric morphometrics to quantify a number of characters used in the species descriptions. Using these methods I demonstrate that most taxa within the genus are distinguishable based on wing pattern and that pattern has evolved along different trajectories between species. I then use Next Generation Sequencing data to distinguish among the populations of *N. mitchellii* and show the two endangered taxa are clearly distinct from other populations of the species. Finally, I apply Approximate Bayesian Computation to compare models of post-glacial range expansion.

ORAL 13:50-14:10

Leptidea Wood White butterflies as an emerging model to study speciation

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Recent findings made the *Leptidea* Wood White butterflies one of the most striking examples of cryptic species in Eurasian butterflies. The three taxa involved (*L. sinapis*, *L. reali* and *L. juvernica*) have recently been confirmed to represent distinct species due to genetic differences and reproductive isolation due to female mate choice. In addition, this butterfly genus displays pronounced karyotype variability and *L. sinapis* is currently the metazoan with the highest intraspecific chromosome number variability (2n=56 to 2n=106), not related to polyploidy. Its chromosomal races seem to follow a longitudinally-oriented cline, making it a perfect case to study the concept of clinal species. Therefore, *Leptidea* is an emerging model to study speciation and is being an increasingly popular research system for several laboratories in Europe. We present new findings related to the chromosome number variability in *L. sinapis* and show that its amplitude is even higher than previously known. Based on laboratory experiments, we also used *L. sinapis* to test the concept of clinal species by mating extreme chromosomal races of this butterfly. Furthermore, through a combination of genitalia morphometrics and DNA data, we were able to detect the first known natural hybrid between *L. sinapis* and *L. reali*. A survey of *Wolbachia* infections across various populations of *L. sinapis*, *L. reali* and *L. juvernica* provided data that could help understand the genetic patterns detected in these species. At present, a genomics approach is being developed with the aim of better understanding the causes of the chromosomal instability and speciation mechanisms in *Leptidea*.

ORAL 14:10-14:30

The assessment of macroecological patterns in butterfly-hostplant associations at a global scale

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Plant-animal associations have been studied in many different contexts in the past, and a great amount of local knowledge has accumulated in several sources and over decades of research. Here we show how available on-line resources facilitate the integration of this knowledge with modern analysis tools in order to improve our understanding of macroecological patterns in biotic associations between butterflies and their hostplants. We found a total of 50,969 records of butterfly-hostplant associations in on-line databases, open-access literature and biodiversity portals, and validated 86% of them with taxonomic and bibliographic tools. The final compilation includes information from 5,152 butterfly species (28.6% of worldwide species of Papilionoidea) and 1,193 genera (66.3%), associated with 6,008 host plant species, from 2,289 genera and 212 families. We summarized the information in association matrices at different taxonomic and geographic levels, and calculated diet breadth and phylogenetic dissimilarity in host species. We used consensus butterfly and plant phylogenies, and summarized distribution records for all butterfly species, plant species and genera in our compilation. We found a significant and strong correlation between host plant diversity and butterfly species diversification at the subfamily level, but there are extreme examples of generalization and specialization at higher taxonomic level. Fabales are used more frequently than any other plant order across all seven butterfly families and most subfamilies, while Poales, the second most frequently used order, is mostly restricted to two species-rich subfamilies. For most butterfly families, diet breadth can explain up to 18% in the variation in the distribution range of the species, but it was uninformative for Hesperiidae and Riodinidae. We also found complex latitudinal patterns in diet breadth, with more specialization in the Neotropics and Afrotropics, but apparently more overlap in resource utilization in the Oriental region.

ORAL 14:30-14:50

A positive relationship between host plant nitrogen content and size of butterflies and moths enhances the observed opposite population trends of species

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One of the large-scale drivers contributing to the ongoing transformation and decline of biological communities is soil nutrient enrichment caused by nitrogen deposition. This driver has previously been linked with changes in vegetation but its impacts on higher trophic levels are less well understood. Here we describe a novel conceptual model that explains how the impacts of nitrogen deposition are mediated through a number of interacting mechanisms which eventually enhance the observed opposite trends of herbivorous insects feeding on oligotrophic or eutrophic plants. As a part of this model, we present (1) a previously unreported positive relationship between host plant nitrogen content and insect size and show how it is linked with other previously reported patterns such as (2) the positive relationship between dietary breadth and insect size, (3) the positive relationship between insect size and dispersal ability, (4) the positive relationship between dispersal ability and the recent population trends of insects and (5) the increasing trends of eutrophic plants (high foliar nitrogen content) and decreasing trends of oligotrophic plants (low foliar nitrogen content) triggered by nitrogen deposition. By using 1,064 butterfly and moth species occurring in northern Europe and representing 11 superfamilies as the focal group we empirically illustrate details of the model. While accounting for the phylogenetic relatedness of species, we show that (1) the size of butterflies and moths is positively and equally strongly related to dietary breadth and mean larval host plant Ellenberg nitrogen (N) indicator value, a proxy for host plant nitrogen content and soil eutrophication, and that (2) the recent (1993–2012) population trends of butterflies and moths in Finland are positively related to dietary breadth of butterflies and moths, their size and the Ellenberg (N) indicator value of their host plants, each variable showing independent explanatory power.

ORAL 15:20-15:40

The rhythm of Africa: dynamics of a fruit-feeding butterfly community

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Long time series of tropical insect communities are needed to interpret results of rapid biodiversity assessments and can provide insights into what regulates populations, and thus help monitor and predict the impact of environmental change, especially climate change. For 14 years, we performed monthly fruit-feeding butterfly trapping at 22 locations in Kibale Forest, Uganda. This is among the longest insect community time series in the tropics, and is complemented with data on species traits and a molecular community phylogeny. Moreover, satellite imagery was used to link the two rainy seasons per year to temporal patterns in vegetation greenness. At the community level, we detected significant seasonality (the first time such pattern was shown for a climate with two rainy seasons per year), and tracking of variation in timing of rainy seasons. However, the interaction between month and year explained the largest portion of the variation, showing that seasonal patterns vary

greatly among years. Nearly all species were at most weakly seasonal: even the one species that is a known seasonal breeder hardly showed seasonal adult abundance. This is likely due to long life spans and wide dispersal. Seasonality appeared to be associated with migrations that are hardly documented. The degree of temporal variation within years, seasonality, and inter- annual variation was not correlated with body size. Irregular abundance fluctuations and rarity of synchrony among (putatively) resident and continuously breeding species suggest that specialist natural enemies such as parasitoids play a dominant role in regulating populations. The results show that seasonality of tropical insect communities cannot be described from a single year, and changes in tropical butterfly communities due to e.g. climate change are hard to detect given the extent of year to year variation. However, responses to inter-annual variation may be used to predict responses to climate change.

ORAL 15:40-16:00

Back to basics: The role of systematics in conservation assessments of butterflies in the Neotropics

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Threatened species programmes were created for and concentrate on vertebrates. There are several impediments to the conservation of hyper-diverse groups such as insects, accentuated in hyper-diverse regions, resulting from issues with taxonomy, quality of data and resources available. The tropical regions present the biggest challenge for taxonomists and conservationists. The abundance and distributional data necessary for a more thorough analysis are unavailable for most species of Neotropical butterflies. Accelerated rates of deforestation in the tropical forests make them a priority for more information and detailed studies on threatened species and future areas for conservation. A systematic study of the poorly-known group of Neotropical butterflies currently placed in the genus *Splendeuptychia* Forster, 1964 (Nymphalidae: Satyrinae) and related taxa is presented as a case study of how well-resolved taxonomies are critical to conservation, given that poorly studied groups may be over-lumped and harbour undescribed species with small distributions. This genus is revised using morphological characters based on data from more than 1,000 specimens and 30 collections around the world, fieldwork, online sources, and literature and in light of recent results from molecular studies. This research examines the feasibility of assessing the threat status of Neotropical butterflies following the current IUCN categories and criteria, using collections data as the primary source and using an accessible methodology which attempts to deal with the challenges of working with poorly known groups. Once unnamed taxa and several cryptic species were recognised, the number of species-level taxa in this group increased significantly, as did the number of threatened species because species with restricted ranges were taxonomically overlooked. This study highlights the importance of detailed systematic research to achieve effective biodiversity conservation. Unless further resources are invested in core areas such as taxonomic studies, threat assessments on hyper-diverse faunas and regions may be biased or inaccurate.

ORAL 16:00-16:20

Biodiversity loss in the Maltese islands hits relictual species

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Because of its biodiversity richness and high endemicity, the Mediterranean basin is considered one of the world's biodiversity hotspots. During the Pleistocene this region encompassed several distinct glacial refugia which generated a mosaic-like structure of different subregions. This created strong genetic contrasts between them and produced biogeographic crossroads characterized by high species richness and genetic diversity. Recent reviews pointed out that Maghreb, Sicily and mainland Italy comprise high genetic variation, while the series of small islands in between could represent important genetic crossroads. The contact zones between North Africa and Europe represent a perfect area to understand the mechanisms shaping the phylogeography of the Mediterranean region. We sequenced the COI gene of the butterfly fauna occurring on all the islands in the Sicilian strait, and compared their genetics with those of populations from Sicily and the adjacent mainland regions. For each species we produced a bi-dimensional representation (PCoA) for mean genetic dissimilarities among areas and combined the results in a final overall representation. We show there is a strong contrast between three main areas: i. Maghreb-Lampedusa, ii. Sicily-Maltese islands, iii. Calabria, while Pantelleria appears to be mostly inhabited by species with low genetic variability. This pattern highly mirrors the Pleistocene paleogeography when Malta was connected to Sicily, Lampedusa to Tunisia, while Pantelleria remained isolated. In particular, the Maltese islands host a series of species genetically identical to Sicily that do not occur on other circum-Sicilian islands. Most of them undergo strong population reductions and in some cases are already extinct from one or both main Maltese islands. Because these species apparently required a land bridge to colonize Malta, they will unlikely recolonize the islands after possible extinction and should be considered as priority species for conservation.

ORAL 16:20-16:40

The gradation from capital to income breeding as an informative way to ordinate lepidopteran life histories

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Providing a classification of life histories may be seen as a key aim of evolutionary ecology. While attempts to apply a single approach to all organisms should perhaps be considered overambitious, such a scepticism does not rule out potential usefulness of more narrowly (in the taxonomic or ecological sense) focussed classification schemes. Indeed, one of such frameworks – the gradation from income to capital breeding – appears to be gaining popularity in insect ecology. While extreme income breeders are critically dependent on adult derived resources in their reproduction, extreme capital breeders do not feed as adults at all. We have suggested that the degree of capital (vs. income) breeding should correlate with a number of other life-history traits at the level of among-species comparison. The primary causal link behind such correlations is suggested to be the trade-off between fecundity and mobility which is characteristic of capital breeders, but not of income breeders. As a result, the females of capital breeders evolve towards limited flight capacity, short adult life spans, indiscriminate oviposition and flight periods early/late in the season. In turn, the larvae of capital breeders are selected to be polyphagous. Until recently, the scarcity of reliable phylogenetic information has precluded testing such hypotheses by rigorous comparative analyses. To fill this gap, we used experimentally derived life-history data, and original phylogenetic reconstructions for the lepidopteran family Geometridae. The degree of capital breeding was estimated on the basis of its morphological correlates, such as the relative size of abdomen in freshly eclosed females. The analyses confirmed most of our theoretically grounded expectations. This indicates that the gradation from capital to income breeding can be used as an informative (i.e., possessing a high predictive power) axis to ordinate life history syndromes in Lepidoptera.

In Lecture hall X

ORAL 16:20-16:40

Homoploid hybrid speciation and genome evolution via chromosome sorting

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Homoploid hybrid speciation (HHS) is usually defined as the formation of a hybrid lineage without a change in chromosome number, and its genetic and ecological mechanisms are highly controversial. In our work, through studying *Polyommatus* (*Agrodiaetus*) butterflies and using method of genomic in situ hybridization, we provide evidence of a previously unknown mode of HHS. First, we demonstrate that species *P. karindus* and *P. morgani* have hybridized in the past, despite pronounced differences in their chromosome numbers. Next, we show that *P. peilei* is their diploid, fertile, and true-breeding daughter species, originated through assortment of the parental species' chromosomes and subsequent fixation of a novel combination of chromosome fusions/fissions. Thus, meiotic segregation, operating in the hybrid lineage, resulted in formation of a new chromosome complement, drastically rearranged in terms of diploid chromosome number, and, consequently, speciation. The work was supported by the Russian Foundation for Basic Research (grant 12-04-00490).

AFTERNOON SESSION: Butterflies as hosts

KEYNOTE 13:30-14:10

Parasite infections, toxic milkweeds and medication behavior in monarch butterflies

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Monarch butterflies (*Danaus plexippus*) are commonly infected with the protozoan parasite *Ophryocystis elektroscirrha*, which strongly reduces monarch survival, mating ability and fecundity. Monarchs are specialized on milkweeds as their larval food plants, and our experiments have shown that milkweed species with high concentrations of cardenolides (secondary toxic chemicals) reduce parasite infection and virulence. We investigated whether monarchs can use medicinal milkweeds to prevent or cure disease, and whether monarch medication behaviors depend on parasite risk in natural populations. Our experiments suggest that infected caterpillars cannot use milkweed as medicine, but that infected female butterflies may preferentially lay their eggs on medicinal milkweeds that make their offspring less sick. Moreover, the type of medication behavior appears to depend on parasite risk in natural populations. In eastern and western North America, where parasite risk is low, only infected monarchs had a preference for medicinal plants, indicating therapeutic medication. In contrast, in South Florida, where parasite risk is high, both infected and uninfected monarchs preferred to lay eggs on medicinal milkweed. Overall, our results suggest that monarchs can use medicinal milkweeds to mitigate the negative effects of their prevalent protozoan parasites, and that the risk of parasitism determines the type of medication that monarchs use.

ORAL 14:10-14:30

The genomic impact of intense selection in a butterfly

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Endosymbionts are increasingly being recognised as important factors influencing many aspects of host biology including host reproduction and sex determination. Sex ratio distorting symbionts are particularly detrimental, yet still common among arthropods. While their presence can produce intense Fisherian selection for the production/survival of the rare sex, evolution of host resistance is rarely observed. Recently, we reported that a dominant host suppressor of a male-killing *Wolbachia*, that enables males to survive infection, had evolved in the butterfly *Hypolimnas bolina*. In one notable case, the suppressor was observed to spread rapidly between 2001 and 2006 on the island of Samoa, evidencing intense selection. In this study, we investigated the genomic impact of the selective sweep in Samoa. Through linkage mapping we first identified the genomic region containing the suppressor. We then examined the pattern of genetic variation linked to the suppressor in samples collected pre- and post- spread. Significant change was observed over a 25cM region surrounding the suppressor locus, making this one of the broadest selective sweeps documented in a natural population. Finer-scale analysis of the region containing the suppressor revealed the presence of a homolog of a *Drosophila* sex-determining cascade gene, doublesex (dsx). In Samoa dsx co-segregates with the suppressor of male-killing, provoking the intriguing hypothesis that the sex-determination pathway is the focus of selection in this system.

ORAL 14:30-14:50

Maternal effects and offspring immune defence in *Pararge aegeria*: implications for dispersal in fragmented landscapes

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Human-modified landscapes are typically characterised by intensive land-use, a decline in habitat quality and an increased habitat fragmentation. Studies on flying insect species have observed changes in movement behaviour and flight morphological traits across differently fragmented habitats and such behavioural and morphological changes have usually been interpreted in an ultimate explanatory context of increased dispersal in fragmented systems. Microparasitic infections can have subtle sub-lethal and persistent effects, such as reducing fecundity and increasing development times. Our recent work has demonstrated that in the Speckled Wood butterfly, *Pararge aegeria* (L.), covert infections can also indirectly affect phenotypic variation in flight morphology and thus adult dispersal ability. In P. aegeria, increased flight during oviposition generates resource-related maternal effects through differential egg provisioning. Offspring from mothers that have increased flight during oviposition have lower larval masses, longer development times and reduced survival after exposure to the viral pathogen *Autographa californica* nucleopolyhedrovirus (AcMNPV). Here I present data examining how flight-related maternal effects and covert AcMNPV infections in offspring interact to influence offspring wing development, and discuss how these complex interactions may influence *P. aegeria* population dynamics, particularly in fragmented anthropogenic landscapes.

ORAL 15:20-15:40

Variation in a host-parasitoid interaction across independent populations

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The antagonistic relationship between an insect host and its parasitoid involves multiple traits in both species. These traits are shaped by their ecological and evolutionary context. The butterfly *Melitaea cinxia* and its parasitoid wasp *Cotesia melitaearum* occur in several populations around the Baltic sea, with differences in landscape structure, population sizes and population histories. We compared the virulence of the parasitoid and the susceptibility of the host from five populations in a reciprocal transplant-style experiment using the progeny of five independent host and parasitoid individuals from each population. The host populations showed significant differences in the rate of encapsulation and parasitoid development rate. The parasitoid populations differed in brood size, development rate, pupal size and adult longevity. Some trait differences depended on specific host-parasitoid combinations, but neither species performed systematically better or worse in experiments involving local versus non-local populations of the other species. The complex pattern of variation in the traits related to the vulnerability of the host and the ability of the parasitoid to exploit the host may reflect multiple functions of the traits that would hinder simple local adaptation.

ORAL 15:40-16:00

Exploitation of butterfly anti-sex pheromones by tiny hitch-hiking parasitic wasps

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Male butterflies are known to transfer species-specific anti-aphrodisiac pheromones to females during mating to enforce female monogamy. Such sexual signal are, however, also vulnerable to chemical espionage by natural enemies.

Here, we show how two closely related parasitic wasp species exploit different anti-aphrodisiac pheromones of two of their butterfly hosts, the gregarious Large Cabbage White *Pieris brassicae*, and the more abundant solitary Small Cabbage White *P. rapae*. Upon detecting an anti-aphrodisiac, the tiny (0.05 mm long) parasitic wasps ride on a mated female butterfly to a host plant and then parasitize her freshly laid eggs (1-6). Interestingly, this behavior is innate in one wasp species, whereas the other learns it after one successful ride on a mated female butterfly. Obviously, parasitic wasps use the sophisticated espionage-and-ride strategy to find eggs of different gregarious and solitary host species. From the wasps' perspective there seems to be a trade-off between the abundance and egg-laying behavior of the butterflies. Besides luring wasps to mated female butterflies, the butterfly anti-aphrodisiacs may also indirectly attract parasitic wasps to butterfly eggs by inducing an indirect plant response when they are transferred onto the plant during oviposition (7,8). Our findings indicate that butterflies can be under selective pressure to minimize the use of an anti-aphrodisiac. References: 1. Fatouros et. al. 2005. Nature. 2. Huigens et. al. 2009. Proceedings of the National Academy of Sciences USA. 3. Huigens et. al. 2010. Behavioral Ecology. 4. Huigens et. al. 2011. Journal of Chemical Ecology. 5. Fatouros & Huigens 2012. BioControl. 6. Huigens & Fatouros 2013. In: Chemical Ecology of Insect Parasitoids (Eds. Wajnberg & Colazza). 7. Fatouros et. al. 2008. Proceedings of the National Academy of Sciences USA. 8. Fatouros et. al. 2009. Journal of Chemical Ecology.

ORAL 16:00-16:20

Inbreeding versus symbiont-induced incompatibility: which of the two for a better conservation strategy?

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Recovery plans of endangered butterfly species often include population supplementation strategies. The captive rearing of larvae collected from large and stable populations, followed by the release of adults in unstable ones, has shown its potential in increasing population size and/or genetic diversity, and consequently in reducing extinction risks. The Oregon Silverspot butterfly, *Speyeria zerene hippolyta*, is officially listed as a threatened species since 1980, and the subject of a long term recovery plan by supplementation since the 90's. Despite all the efforts, the species is only just showing signs of a slow recovery and remains of high conservation concern. Furthermore, several strains of the endosymbiotic bacterium *Wolbachia pipientis*, inducing cytoplasmic incompatibility (CI) in crosses between butterflies from different populations, have been detected in this species. The reduction of host fecundity due to *Wolbachia* increases the risks of extinction in the supplemented unstable populations and reduces the chances of success of the current conservation program. Using inbred and outbred crosses, we propose to compare the levels of inbreeding depression and CI, thus informing on the best approach for the Oregon Silverspot butterfly conservation program.

THURSDAY 14.8.2014

MORNING SESSION: Systematics and Diversity

PLENARY 09:00-10:00

Butterfly biodiversity and operational species

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Butterfly biodiversity, with species as the basic unit, has long been fundamental to conservation, ecology and evolutionary biology. But agreement on what those butterfly species actually are has been frustratingly slow, and names lists have remained a source of divisiveness among the diverse communities of their users. I will explore several examples of North American butterfly species names from the viewpoints of different user groups, with the aim of determining whether it is realistic to make the identification and delimitation of these species more operational. A variety of operational trends has swept through, ranging from genitalia dissections to field marks to DNA barcoding, with each wave contributing toward resolving some questions while raising new ones in a series of Bayesian iterations. Such operational cycling is the essence of hypothesis testing, although the question remains whether butterfly species even exist independently of our perceptions. I will argue that a partial solution to that question is to frame species in terms of the maintenance of genomic integrity. Nonetheless, the quest for consensus on the application of butterfly species names may remain as elusive as agreement on what we need those names for.

ORAL 10:30-10:50

Morpho, *Bia* and biogeography (Nymphalidae, Satyrinae)

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While *Morpho* (Morphini) includes 29 species that collectively range from Mexico through Central and South America, *Bia* (Brassolini) currently contains only two strictly Amazonian species. Work in progress revealed several *Bia* entities that are distinguishable through wing color and androconial characters (C. Penz, M. Casagrande, P. DeVries & T. Simonsen, unpublished), and although two undescribed *Bia* co-occur with others, most of these entities are allopatric. Dated phylogenies available for *Morpho* and the Nymphalidae, information about species distribution, and a recent demographic model allowed us to compare *M. achilles* and *Bia* and ask questions that have broad implications for the biogeography of our focal butterflies and many others.

ORAL 10:50-11:10

Testing the Red Queen and the Court Jester Hypotheses: The role of ecological and evolutionary determinants in the radiation of Apollo butterflies (Papilionidae: Parnassiinae)

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Biodiversity dynamics is mostly influenced by biotic factors (intrinsic to the species, ecological traits) as in the Red Queen hypothesis or abiotic factors (extrinsic to the species, evolutionary events) as in the Court Jester hypothesis. We tested the contributions of each hypothesis using the Apollo butterflies (Papilionidae: Parnassiinae) with a phylogenetic approach. We reconstructed their phylogenetic relationships based on a specimen-level dataset in order to retain a clarified taxonomic framework for estimating their age and divergence times using Bayesian inferences with five genes and three fossil calibrations. The resulting chronograms allowed studying the diversification processes using various birth-death models. The Red Queen was assessed by testing the impact host plants had on the diversification, and testing for diversity-dependence pattern involved by niche filling and competition. The Court Jester was investigated by testing whether rate shifts occurred during major environmental changes (Himalayan rise, climate change), testing whether the altitude had an effect on the diversification and whether the paleotemperature positively or negatively influenced the diversification of the clade. Strongly supported and resolved phylogenetic trees were recovered resulting in a 99% complete chronogram. We found that the Parnassiinae diversified in the late Eocene whatever the fossil calibrations and priors were used. Diversification analyses indicated that both the Red Queen and the Court Jester significantly impacted the diversification of the Parnassiinae. In particular host-plant shifts always favored high speciation rates, in agreement with the escape and radiate hypothesis. Meanwhile we did not support diversity-dependence but found that shifts in diversification occurred in the late Pliocene and that diversification is positively correlated to paleotemperatures, which indicate that the Parnassiinae diversified better during warm climate. Altogether the interplay between parnassiine's ecology and climate change are the main drivers of their diversification.

ORAL 11:10-11:30

Systematics, ecology and diversity of ithomiine butterflies

Elias, Marianne(1,4), Willmott, Keith R.(2), Freitas, André V. L.(3), Chazot, Nicolas(4), de Silva, Lisa(4)
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Ithomiine butterflies (Nymphalidae: Danainae, Ithomiini), which are found from sea-level up to 3000m and numerically dominate forest butterfly communities, form one of the most fascinating groups of butterflies in the Neotropics. All of the 380 constituent species engage in chemically-defended, mutualistic Müllerian mimicry and have aposematic colours. At the same time, the wings of many ithomiine species are transparent, a striking feature rarely found in true butterflies. Over the last couple of decades, there has been on-going collaborative effort to refine higher and species-level systematics and to explain the exceptional diversity of the tribe. Phylogeny-based analyses of diversification and community structure in multiple Ithomiini lineages suggest that mimicry, hostplant adaptation and the geological history of the neotropics have all contributed to the evolutionary success of the Ithomiini. In addition, community ecology analyses show that mutualistic interactions are powerful enough to adaptively shape species assemblages by driving ecological convergence in multiple niche dimensions. Future projects should focus on the processes acting at a microevolutionary scale, such as the role of mimicry in reproductive isolation, to better understand diversity patterns revealed at a macroevolutionary scale.

ORAL 11:30-11:50

Cryptic matters: the relevance of hidden butterfly diversity

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The descriptive taxonomical task that we biologists are facing is enormous, yet pressing. Technical advances, especially recent molecular techniques, are unfolding a previously unsuspected cryptic fraction of biodiversity: multiple species that were lumped in a single one due to their morphological similarity. Thus, taxonomical discovery is now noticeably split in two fronts: the pursuit of species not yet studied in most remote habitats and the search for unrecognized species in modern laboratories. Even if butterflies are an exceptionally well studied group of invertebrates, a good number of cryptic species are being discovered or confirmed. I will discuss about wide-scale molecular methods as a way to highlight potentially unrecognized species and show the results of a first cryptic biodiversity assessment for Europe. However, species status can only be convincingly demonstrated through detailed studies combining multiple

sources of data. In fact, unexpected layers of hidden diversity keep on appearing when using increasingly refined approaches. Is describing cryptic diversity relevant beyond the sheer number of species? It obviously is for conservation purposes and in order to avoid an anthropocentric view of nature. Indeed, cryptic diversity tells us a lot about the limitations of our own perception, but also about evolutionary and speciation processes. This fraction of biodiversity comprises original qualitative aspects in addition to merely quantitative ones. As a result, including cryptic diversity in wide-scale surveys and in ecology and biogeography studies is necessary to avoid important biases.

ORAL 11:50-12:10

Phylogenomics using nearly 2700 genes provides strong evidence for relationships of butterflies and moths

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Butterflies are one of the most charismatic and well-known insects. Together with moths, the order Lepidoptera comprises ˜160,000 described species, including many important model organisms. Previous studies on the evolution of Lepidoptera did not provide confident placement of butterflies. Relationships among major moth superfamilies, especially those among the megadiverse clade Ditrysia, also remain largely uncertain. We addressed these problems by identifying 2,696 single copy genes common among all Lepidoptera reference genomes. We sequenced 33 new transcriptomes and combined these data with 13 available genomes, transcriptomes and ESTs. We conducted a next-gen superalignment and created a dataset of 46 taxa that represent the majority of key superfamilies of Lepidoptera. Nucleotides and amino acids of the all-gene, all-taxon dataset yielded nearly identical, well-supported trees. Monophyly of butterflies (Papilionoidea) was strongly supported, and the group included skippers (Hesperiidae) and the enigmatic butterfly-moths (Hedylidae). Butterflies were placed at the base of the Obtectomera and the remaining obtectomeran Lepidoptera were grouped with 87% bootstrap support. Establishing confident relationships among the four most diverse macroheteroceran superfamilies were previously challenging, but we recovered 100% bootstrap support for the following relationships: ((Geometroidea, Noctuoidea), (Bombycoidea, Lasiocampoidea)). We present the first robust tree of Lepidoptera that contradicts historical placement of butterflies and provide an evolutionary framework for genomic, developmental, and ecological studies on this diverse, popular insect order.

AFTERNOON SESSION: Evolutionary phylogenetics

KEYNOTE 13:30-14:10

What is host range? (happy meetings between phylogenetic and experimental studies)

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During the last decades, the application of phylogenetic methods to problems regarding species interactions, trait evolution and diversification has been a continuing success story. Methodological development has allowed for ever more sophisticated questions to be asked. Yet, there are, and always will be, limitations to the types of questions that can be answered with strictly phylogenetic methods. I will here make a case for the fruitful combination of phylogenetic comparative investigation with experimental methods on physiology, life history, behavior and genomics. I will use our several decades long investigation of the host plant associations of the Nymphalid butterflies to show how we try to get at the mechanisms behind host range evolution, but also how a comparative mindset can help guiding experimental work to ask more interesting questions.

ORAL 14:10-14:30

Asynchronous divergence of *Cymothoe* forest butterflies and their *Rinorea* host plants in tropical Africa

van Velzen, Robin(1), Onstein, Renske E.(2), Sosef, Marc S.M.(3), Bakker, Freek T.(1)
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Insect herbivores are generally highly host specific and insect-plant associations seem phylogenetically conserved, with related insects usually feeding on related plants. However, the factors that generate, maintain, and constrain these associations remain unclear. We investigate the system of *Cymothoe* forest butterflies (Nymphalidae, Limenitidinae) and their *Rinorea* host plants (Violaceae) in tropical Africa, which is especially suitable for untangling processes shaping patterns of host plant associations because of its high level of specificity (mostly monophagous) and the high number of related species involved (33 herbivores and 32 hosts). Our aim was to distinguish between alternative scenarios for the evolution of insect-host plant associations by comparing species-level phylogenetic trees of *Rinorea*-feeding *Cymothoe* with that of their hosts within an absolute time-frame. Our results show that: (1) Divergences among extant *Cymothoe* are more recent than those among their associated *Rinorea* hosts, suggesting asynchronous diversification of *Cymothoe* herbivores onto already diversified clades within African *Rinorea*. (2)

Phylogenetic trees of *Cymothoe* and their associated *Rinorea* host plants are discordant and current associations between *Cymothoe* herbivores and their *Rinorea* hosts have developed primarily through a process of host shifting rather than by cospeciation. (3) Related *Cymothoe* tend to feed on related *Rinorea* hosts. Based on the available data, we propose a recent origin of *Rinorea*-feeding by *Cymothoe* butterflies with a single colonisation of pre-existing lineages in the late Miocene. We find no support for reduced diversification of African *Rinorea* after the colonisation. Current associations are best explained by a predominance of shifts among related plants, probably due to constraints in larval physiology and female oviposition behaviour. These findings are in agreement with a scenario of sequential evolution as a dominant pattern in insect-plant interactions.

ORAL 14:30-14:50

Spectacular radiation of Mycalesina butterflies in the old world tropics

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The radiation of the species rich (ca. 300 species) butterfly group, mycalesina, in the Old World tropics is not only spectacular but also presents an excellent platform for investigation in detail the pattern and processes of adaptive radiation. Using DNA sequences of nine gene regions of over 200 exemplar taxa, we have reconstructed a phylogeny and historical biogeographic hypothesis for the mycalesine butterflies. We identified Asia as the ultimate origin of the subtribe in the early Oligocene (˜37 MYA). Africa is believed to have been colonized at least twice by Asian mycalesines between 33-28 MYA. The first colonisation event was by ancestors that gave rise to *Heteropsis* on mainland Africa, which most likely went on into Madagascar via oceanic dispersal from Africa and back colonise Asia. The second colonisation event also in the Oligocene resulted in the African genera *Hallelesis* and *Bicyclus* on mainland Africa, which could neither back-colonised nor dispersed successfully to Asia or Madagascar. The *Mycalesina* radiations which responded to or exploited new ecological opportunities, following dispersal into new regions (i.e. *Bicyclus* in Africa, *Heteropsis* in Madagascar and *Mydosama* in Indo-Australia) had been rapid, similar to an early burst of evolutionary diversification and this observation is supported by the gamma statistics and best fit diversification model analyses. This gives us the first hint that the radiations within mycalesine could be adaptive, possibly involving invasion into new ecological niches and ecological speciation. It is interesting all these rapid radiations are dated between 20 and 10 MYA, coinciding with the period of warmer climatic conditions and major grassland expansion in the old world tropics, thereby implicating climate and host plants shifts as a possible drivers of Mycalesina diversification.

ORAL 15:20-15:40

Effect of vertical stratification in the phylogenetic structure of a fruit feeding butterflies community of an Atlantic Forest, Brazil

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Spatial scaling of phylogenetic diversity (PD) can be driven by evolutionary and ecological process such as environmental filtering, dispersion limitation and inter or intra specific interactions. Tropical forests present a wide variation in the complexity of vegetation structures along the vertical dimension, resulting in different microclimates that influence the vertical distribution of several animal groups. If the vertical gradient plays a role in relation to phylogenetic structure of communities, we could expect species traits being adapted to this gradient. So, species with more similar morphological traits or niche will tend to co-occur in the same habitat, fitting in a non-random distribution of clades along the vertical gradient. We tested the effect of vertical gradient of conditions in the phylogenetic structure of community of fruit-feeding butterflies. Additionally, we tested the wing size as a functional trait and if this is being selected by this gradient or if the phylogeny are mediating the relation of this trait with the habitat. The community of fruit-feeding butterflies presented differences in the phylogenetic arrangement between strata, with lineages of Charaxinae more associated to the canopy, unlike the remaining subfamilies, related to understory. Besides that, we found a strong phylogenetic signal for wing size, confirming it as conserved within clades. Phylogenetic diversity was higher in the understory and decreased towards the canopy. Wing size differed between strata, with larger butterflies found in the lower levels, however this difference was not mediated by phylogeny, but due to an effect of a different process. The presence of a phylogenetic signal for one functional trait does not necessarily predict conservatism niche in this case. Other selective forces such as pressure of predation or the ability to fly in reduced spaces may be important to determining different wing sizes along the vertical gradient, independent of phylogenetic structure.

ORAL 15:40-16:00

Diversification in the Neotropics: insights from Ithomiini butterflies

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The biodiversity of the Neotropics has long fascinated biologists. Understanding the factors and processes underlying this diversity is key question in evolutionary biology. This question has been subject to a still ongoing debate. Many hypotheses have been proposed and most recently focus has been given on the role of the Andes. However most studies have been conducted on vertebrates and plants, and results are still lacking for other groups. Ithomiini butterflies are probably one of the best-studied groups of Neotropical butterflies. They have received many attentions through systematic, phylogenetic, biogeographic and community studies, allowing to draw relatively precise pictures of the history of this group. Ithomiini are highly diverse, especially along the Andes. Throughout all the Neotropics they occupy forest habitats from lowlands up to 3000m high. They are all involved in Müllerian mimicry interactions, which have lead to a particular evolution of their colour patterns. In this work we test several hypotheses in order to understand diversification dynamics of diversification through time and space in this group. We also try to understand what drive the pattern of high species richness in the Andes found in many groups by contrasting hypotheses such as the age of colonisations, differential rates of diversification and rates of colonisation. Instead of classical hypotheses of montane museum vs species pump , we argue that multiple independent colonisation events play a major role in this pattern of diversity, and may be explained by the most recent uprising of Northern Andes in the last 10 million years.

ORAL 16:00-16:20

An alternative, plant-based time-tree implies conflicting dates for the diversification of ithomiine butterflies (Lepidoptera: Nymphalidae: Danainae)

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The recent publication of a time-tree for Solanaceae (nightshades) provides the opportunity to use independent calibrations to test divergence times previously inferred for the diverse Neotropical butterfly tribe Ithomiini. Ithomiini includes species that are obligate herbivores of Solanaceae, with some genera feeding on only one genus. We used eight calibrations extracted from the plant phylogeny under a relaxed molecular-clock analysis to produce an alternative temporal framework for the diversification of this group of butterflies. We compared the resulting age estimates to: (1), a time-tree obtained using three secondary calibrations from the Nymphalidae tree of Wahlberg et al. (2009), and (2), Wahlberg et al.'s (2009), original estimations. Secondary calibrations obtained from nightshades produced mean age estimates on average 57% younger than previous hypotheses. The implications of using these alternative secondary calibrations are discussed in light of ithomiine natural history and biogeography.

ORAL 16:20-16:40

Evolution and biogeography of Batesian mimetic diversity in *Elymnias* butterflies (Nymphalidae: Satyrinae)

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The genus *Elymnias* is a little-studied group of mimetic butterflies that vary dramatically in color, pattern, and size because different species frequently mimic dramatically different models. Sexual dimorphism—where the female mimics one model species and the male mimics another—is also common. Most of the ~50 species have diversified across the geologically complex Indo-Australian Archipelago, which comprises more than 20,000 tropical islands. We sequenced seven molecular markers from ~150 specimens representing all known *Elymnias* species to reconstruct a species tree of the group and infer divergence times. Similar wing patterns have evolved convergently multiple times, and sexually dimorphic wing patterns have also evolved multiple times. In situ speciation has occurred only on the Asian mainland and on the large islands of Sulawesi and New Guinea. When two or more endemic *Elymnias* are found on smaller islands, the species are from distantly related lineages. The propensity for convergent phenotypic evolution among *Elymnias* species and recent work on the developmental genetics of butterfly wing patterns suggests that a limited number of developmental genes might be involved in generating the tremendous morphological diversity that characterizes the genus.

ORAL 17:00-17:20

Nature's ecological recorders - stable isotopes reveal patterns of ecological diversification in Mycalesine butterflies

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Mycalesine butterflies have radiated dramatically in Africa, Madagascar and Asia to produce more than 300 extant species. Larval host plants are nearly always grasses. The primary driving process of these radiations could have been the ability of mycalesine butterflies to invade the empty niches that arose as a result of the evolution of the C4 photosynthetic pathway and the world-wide expansion of C4 grasses in open, sunlit environments. Primary forest species of mycalesines are expected to be C3 specialists as the advantage of the C4 pathway declines in shaded forest understories where cool conditions improve the relative photosynthetic efficiency of C3 grass species. In more open habitats we expect mycalesines to be more opportunistic and generalist in their host plant choice or even to have become C4 specialists. Reliable host plant data are essential to be able to test this hypothesis but detailed host plant records for mycalesines are very limited. However, over the last three decades stable isotope analyses have become an important part of the ecologist's toolbox. Here, we used daily trap captures of adult butterflies from a community of three sympatric species of *Bicyclus* in Malawi to explore whether 13C values can be used to detect micro-climate conditions during the larval development of Mycalesine butterflies. In addition, we have begun to examine the larval feeding preferences, in terms of C3 and C4 grasses, in a broader phylogenetic-ecological framework. By mapping the data of this large scale isotope survey and the current habitat preferences of *Bicyclus* butterflies onto the phylogenies we are now able to reconstruct whether one or more shifts to C4 grasses were associated with the colonization of open habitats and subsequent expansions.

ORAL 17:20-17:40

Higher-level phylogeny and biogeography of the Riodinidae

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We present the first dated higher level molecular phylogeny and biogeographical analysis of the 'metalmark' butterflies, the family Riodinidae. This family is distributed world-wide, but more than 90% of the approximately 1500 species are found in the Neotropics, while the some 120 species of Old World taxa are mainly concentrated in Southeast Asia with only a few species in other regions. Classification within the family based on morphology has been difficult and many genera are not currently assigned to tribes, or only tentatively so. Using 3667 bp from one mitochondrial and four nuclear markers for each of 123 taxa representing all subfamilies, tribes, subtribes and 96 out of 145 described genera of Riodinidae as well as 22 outgroups, we find that Riodininae split from Lycaenidae about 96 Mya in the mid Cretaceous. The family is recovered as monophyletic and most likely originated in the Neotropics. Neither Euselasiinae nor Nemeobiinae are monophyletic as currently circumscribed. Within Riodininae our inferred relationships also differ from the currently accepted morphological hypothesis. We find that Eurybiini is the sister group to the rest of the subfamily followed by Mesosemiini, and none of the subtribes within tribes Nymphidiini and Riodinini are monophyletic. The historical biogeography of the family turns out to be difficult to infer for current methods, and this is especially the case for the enigmatic monotypic genera *Styx* and *Corrachia*.

ORAL 17:40-18:00

Developmental and evolutionary consequences within the lepidopteran wing scale morphospace

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We have created the first comprehensive morphospace that analyzes the range of lepidopteran wing scale shapes in light of their evolutionary relationships. Much of a butterfly's life plays out through the coloration of its wings, and those colors are facilitated or produced by the patterning on scale cells. The architectural complexity of wing scale shapes and colors arises from differences in the developmental program of just a single cell type. Significant advances have been made in understanding the developmental underpinnings that generate lepidopteran wing patterns, but researchers still understand very little about the formation of a wing scale. Previously, we have shown that F-actin bundles play a major role in wing scale elongation, and that the arrangement of actin bundles produces some of the morphological features (such as longitudinal ribs) seen in butterfly wing scales. In order to place the cytoskeletal work into a broader context, we have set the range of scale forms (including their longitudinal ribs) in a morphopace that surveys wing scales collected from homologous wing regions of 40 of the 48 superfamilies (and 130 genera) of Lepidoptera. Fourier transformations of outlines of the scales were used for principle component analysis. We have built a maximum likelihood tree, constrained to the families in the morphospace. By connecting the space to a phylogeny, we assess patterns of evolutionary convergence as well as the emergence of novel shapes on branches of an evolutionary tree. The number of longitudinal ribs that run along a scale are 1:1 representations of the number of actin bundles in the developing wing scale cell. Thus features of wing scales (e.g. number of finger-like projections, scale width, scale length) can be connected to processes occurring during scale cell development in the pupa.

AFTERNOON SESSION: Behavioural ecology

KEYNOTE 13:30-14:10

Neuroethology of monarch butterfly migration

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Studies of the spectacular migration of the eastern North American monarch butterfly have revealed mechanisms behind its navigation using a time-compensated sun compass. Skylight cues, such as the sun itself and polarized light, are processed through both eyes and integrated through complex circuitry in the brain's central complex, the presumed site of the sun compass. Circadian clocks that reside in the antennae and have a distinct molecular mechanism provide time compensation. Recent evidence suggests that migrating monarchs can also use a magnetic compass for orientation in the absence of skylight cues. Genomic and genetic strategies have been developed to manipulate the monarch genome to access the genetic underpinning of the migration. The monarch butterfly has emerged as a tractable system to study the neural and molecular basis of long-distance animal migration.

ORAL 14:10-14:30

Perceptual biases and visual attraction: The role of perception in reproductive isolation

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Sexual selection has resulted in the evolution of elaborate and diverse secondary sexual traits in multiple taxa. This diversity in sexual ornamentation is often associated with diversity in mate preferences for these sexual ornaments, which has the potential to influence reproductive isolation and speciation. While variation in sexual ornamentation has been extensively studied in multiple taxa, little attention has been given to mechanisms underlying the variation in preference that must accompany the variation in ornamentation in order to maintain assortative mating. Here, we use *Heliconius cydno* butterflies to decipher the molecular genetics and neurobiology associated with a change in mate preference for a visual signal. White and yellow *H. cydno* males mate assortatively and are attracted to a single, specific color (white or yellow, depending on the male) on female wings. In addition, females do not have complex visual displays. This makes *H. cydno* an ideal system for studying changes in perception associated with visual attraction, because we have a cleaner shot at finding input processing differences and an accompanying set of associated genes than in most other visually sexually motivated organisms (whose sexual displays consist of multiple colors or multiple movements). Using whole genome sequencing we identify a regulatory region perfectly associated with the relevant visual attractant, and explore the association between this regulatory region and mate preference for the visual attractant. In order to determine the neurological underpinnings of visual perceptual biases associated with mate attraction, we use electrophysiology experiments to compare the spectral tuning of the eyes of males who prefer white to those of males that prefer yellow on the wings of females. We discuss our results in the context of perceptual biases, the role of behavior in maintaining morphological diversity, and the value of butterflies as model systems for sexual selection and visual perception.

ORAL 14:30-14:50

Heliconius mimicry rings in a new light: Light environment and signaling

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Although mimetic coloration has been studied since Darwin's time, many questions on the efficacy, evolution, and function of mimicry remain unanswered. Müller (1879) hypothesized that unpalatable individuals will converge on the same conspicuous coloration to reduce predation risk. However, there are many cases where closely related, unpalatable species have diverged from a shared conspicuous pattern. What is the adaptive value of the divergence of warning colors? Environmental factors such as ambient light (irradiance) have been hypothesized to affect signal transmission and efficacy in animals. We test this light-environment hypothesis using aposematic *Heliconius*butterfly mimicry rings. Different mimetic groups are segregated by microhabitat and most likely by light environment. We demonstrated that mimicry rings differ in the light environments where they occur and that different light environments affect ring color conspicuousness of butterflies. Furthermore, we showed that butterflies preferred specific light environments in which they are conspicuous and how the visual systems of these mimetic butterflies correlates with light environment. Through this research, we better understand the effects of light environment on visual signals and systems. We also demonstrate the functional value of divergence in Müllerian mimics and mimicry rings as well as the diversity of butterfly coloration as it relates to environmental factors and signaling.

ORAL 15:20-15:40

Nutrition as a constraint on brain and life history evolution: a comparative study across butterflies

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The availability of energy and nutrients has been hypothesized to be an important factor in limiting the evolution of large brains and other fitness-related traits. However, organisms readily adapt to low nutrient environments, calling into question whether diet can be a strong evolutionary constraint. Butterflies offer an attractive system to tackle this question because larval nutrition is relatively consistent within species, highly variable across species, and phylogenetic approaches can be used to estimate how long a species has been feeding on their current diet. Here I present data from over 60 species across five butterfly families testing how nitrogen and sodium availability shape life history traits and neural investment (relative eye size, total brain size and the size of neuron-dense brain regions). Nitrogen availability appears to be particularly important in the evolution of neural and life history investment, even across species that have been feeding on their diet for over 50 million years. For instance, species that feed on higher nitrogen host plants have larger relative eye size, more (but smaller) eggs, and larger body size. Sodium availability and the timing of a diet shift are important correlates of neural investment and fecundity within some lineages. We also use this data set to test for brain-life history tradeoffs across species. Overall, these results provide support for the idea that nutrition can shape the evolution of not just brains, but life history investment more broadly.

ORAL 15:40-16:00

Ecological genetics of life history and host plant adaptation in the Glanville fritillary butterfly

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To understand how evolutionary adaptation works in nature, it is essential to uncover the genetic basis of adaptive traits and to take into account the ecological conditions that determine variation in fitness in wild populations. Using the well-studied Glanville fritillary butterfly metapopulation of the Åland Islands in Finland, we measured life history traits and female host plant preference of wild butterflies and their offspring in a large outdoor population cage, and associated trait variation with SNPs in candidate genes. The genes were selected on the basis of previous genomic and transcriptomic studies and have been linked to the physiology and life history of this or other arthropod species. We discovered a strong association between genetic polymorphism in the cytochrome P450 gene CYP337 and lifetime reproductive success, a key component of fitness. This gene belongs to a group of cytochrome P450 genes that have a well-documented role in host plant adaptation in Lepidoptera and other insects. Previous research has shown that the Glanville fritillary Åland population exhibits spatial variation in host plant adaptation involving two plant species, *Plantago lanceolata* and *Veronica spicata*. We show that the CYP337 genotype is associated with host plant preference and that there is a strong correlation between CYP337 allele frequency and host plant preference across the Åland Islands. Our results suggest that the spatial heterogeneity of the habitat may contribute to the maintenance of genetic variation in CYP337 through local adaptation.

ORAL 16:00-16:20

Specialist and generalist oviposition strategies in butterflies: maternal care or precocious young?

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Herbivorous insect species specialized on a narrow set of plants are often believed to be better adapted to their specific hosts. Indeed, species with a broader host plant range have in many cases been shown to pay a cost through decreased oviposition accuracy. Despite a wealth of studies investigating oviposition behavior there is a lack of knowledge on how larvae can cope behaviorally with their mothers' egg-laying strategies. Low oviposition accuracy should select for larvae that are more mobile and with a higher ability to locate favorable feeding sites of their own, i.e. they should be more precocious. We examined neonate larval behavior of four nymphalid butterfly species with different host plant ranges in several disadvantageous situations that are likely results of female oviposition mistakes. We found that larvae were more mobile and survived better in unfavorable situations if their mothers had a broader host plant range. Intriguingly, the two generalist species *Polygonia c-album*and *Vanessa cardui* differed in several aspects, such as larval survival, growth rates, egg weight and egg output. This suggests that polyphagy is not a uniform condition across species, but can be achieved through different suites of traits, probably reflecting different evolutionary histories.

ORAL 16:20-16:40

Genetic and environmental constraints on protectiveness of coloration: a genetic trade-off between efficacy of adult and larval warning coloration in an aposematic moth

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If predators select conspicuous warning signals that facilitate learning and memorability, then the higher survival of prey individuals bearing them should rapidly exhaust genetic variation for conspicuousness and drive traits to fixation. Still, high genetic and phenotypic variation is common among aposematic animals. Genetic variation in signal expression is claimed to be predominantly maintained by genetic trade-offs between the signal efficiency and fitness traits within one life stage. Here, we tested the alternative and novel hypothesis that warning signal evolution is genetically constrained when different life stages share a common resource pool, in this case proteins allocated to colour pigments. We showed that having an effective primary defence as a larva results weaker defence as an adult, due to the negative genetic correlation between larval and adult female warning colouration in the wood tiger moth (*Parasemia plantaginis*). Furthermore, the conspicuousness of the warning signal (i.e. low level of melanism) of larvae was positively genetically correlated with high growth rate, suggesting low costs of producing and maintaining a bright warning signal and high costs of melanin. This costly melanin hypothesis was then tested experimentally by rearing larvae from high and low melanism selection lines on low and high protein content diets. Production of genetically determined melanic coloration was costly, as larvae from the high melanism selection line had lower immunological responses on low protein content diet than on high protein diet. Individuals were also darker in general on the high protein diet than on the low protein diet. Altogether, our results demonstrate that by analysing the trait variation in relevant life-stages and in different dietary conditions, we can determine the principal mechanisms maintaining genetic and phenotypic variation in traits under strong directional selection.

ORAL 17:00-17:20

Relative effectiveness of blue and orange warning colors at deterring naïve avian predators

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Warning colors can deter predators from attacking unpalatable or toxic prey. These colors are avoided by predators, either innately or by learned response through trial and error. Predators that have learned these signals can also generalize their responses to similar colors. Much of the previous research on warning colors has focused on their long-wavelength components (e.g. orange and red) and little is known about short-wavelength components (e.g. blue), although they are common in unpalatable animals in many different taxa, including the unpalatable Pipevine Swallowtail butterfly (*Battus philenor*). *B. philenor* displays both an iridescent blue coloration and orange pigment-based coloration as part of the ventral surface warning signal. We determined the relative effectiveness of these colors at deterring predation by a naïve avian predator (Gambel's quail chicks, *Callipepla gambelii*). The prey in our experiments were pieces of mealworm (palatable or unpalatable) beneath a paper tent with B. *philenor* wing pieces attached. Blue alone did not elicit any innate avoidance, whereas orange and a combination of both colors (orange-and-blue) deterred naïve predators. Predators learned the association between color and unpalatability most effectively for orange prey, but also learned this association for blue and for orange-and-blue prey. Predators exhibited asymmetrical generalization, in which birds were more likely to generalize from the color they learned as unpalatable (conditioned stimulus) to orange prey and orange-and-blue prey than to blue prey. We conclude that orange is likely the most effective component of the multi-component warning signal of animals like *B. philenor*. However, blue can still be learned alone and when displayed adjacent to orange, and may provide a back-up signal under variable environmental conditions.

ORAL 17:20-17:40

Heritability of bright coloration in the Pipevine Swallowtail (*Battus philenor*)

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Two primary contexts for the adaptive evolution of bright coloration are competition for mates (i.e. mate choice) and avoiding predator attacks (i.e. warning coloration). Bright animal coloration can be iridescent, in which the surface appears to change color with changing viewing or illumination angle. Bright animal coloration can also be produced by pigments, which do not appear to alter color with changing viewing or illumination angle. The Pipevine Swallowtail, *Battus philenor*, is unique in having both sexual signals and warning coloration that include iridescent and pigment components, both of which are variable in color. The aim of our study was to examine the role genes play in producing this variation, providing us a sense of potential indirect benefits of female choice. We tested the hypothesis that color variation has a genetic component. We predicted that in a full-sib analysis there should be greater variation in the coloration of the sexual and warning signal among families than within families. We reared *B. philenor* under standard laboratory conditions and analyzed heritability using a full-sib analysis. We collected reflectance measurements for components of the sexual and warning signal iridescence using a spectrophotometer and used CLR (color analysis software) to extract brightness, hue, and chroma values. We used a multivariate ANOVA (IBM SPSS, v. 21) to analyze the warning signal variation, and a generalized linear mixed model (IBM SPSS, v. 21) to analyze the sexual versus warning signal variation in males. A significance value of 0.05 was used for both analyses. Our results indicated a genetic component to coloration, implicating indirect benefits in *B. philenor* female mate bias. Further research on bright coloration in *B. philenor* indicates that there may also be direct benefits of female mate choice.

ORAL 17:40-18:00

The erroneous courtship hypothesis: do butterflies really engage in aerial wars of attrition?

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Males of various flying insects perform conspicuous aerial interactions around their mating stations. The broadly accepted interpretation of their aerial interaction is a war of attrition, where two contestants inflict costs on themselves by performing display and the one that reaches its cost threshold earlier is the loser. This notion is somewhat curious because if display imposes cost not on their opponents but on themselves, exhibiting no display and merely staying in the territory is clearly advantageous. Wars of attrition via costly display do not seem to evolve under Darwinian natural selection. If wars of attrition can evolve, there must be some selective pressure towards display. In contests where physical attacks occur if contests are not settled during the display phase, the pressure is rather obvious. Individuals that do not exhibit display should be physically attacked by their opponent. However in contests where physical attacks never occur, it is difficult to understand why contestants should exhibit display. In addition, the war of attrition model requires insects to distinguish the sex of flying conspecifics; however, they do not necessarily have such abilities. We reviewed research on territorial contests of butterflies and dragonflies to examine the validity of the war of attrition concept. In butterflies, we could not find any evidence that contests involve clear physical attack. Their ability to perform sexual discrimination is also limited. There are thus few reasons to regard butterfly contests as wars of attrition. Their aerial interactions are better interpreted as erroneous courtship between sexually active males. In dragonflies, we found an abundance of evidence that contests involve physical attack and the ability of sexual discrimination is high. Therefore, wars of attrition may occur in at least some dragonflies. We discuss future directions of research on contest behavior of flying insects.

POSTER SESSION: Evolutionary ecology

POSTER 1.

Investigating olfactory communication in the bush brown butterfly *Bicyclus anynana*: selecting reference genes for quantitative real-time expression analyses.

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The butterfly *Bicyclus anynana* is emerging as a suitable model system to study the genetic mechanisms controlling olfactory communication. Transcriptomes from eight tissues corresponding to different morphological structures involved in olfactory communication were sequenced using 454-sequencing method to identify candidate genes involved in olfactory communication. To validate the expression of candidate olfactory genes selected from transcriptome data, quantitative Real Time PCR (qRT- PCR) was used to identify suitable reference gene(s). Finding a set of reference genes for normalizing the transcript abundances of genes of interest is a pre-requisite to any solid conclusion. Amino acid sequences of 12 potential reference genes selected from different lepidopterans were used as a query sequence for a TBLASTN search of the *Bicyclus anynana* genome database. Selected candidate reference protein sequences were then used to query the non-redundant protein database using BLASTX for confirming the identity of proteins. Eight different tissues involved in olfactory communication were used as samples for monitoring the expression of the candidate reference genes in normal and stress conditions using qRT-PCR. The candidate reference genes encoded cytoskeletal proteins (actin and -tubulin), protein cycling process (ubiquitin), translation related proteins (initiation factor IF 4, elongation factor EF 1), stress related protein (Heat shock protein), proteins involved in carbon metabolism (GAPDH, G6PDH) and ribosomal proteins (RPS 8 and RPS 18). Two previously reported reference genes (FK506 and VhaSFD) were also included in the analysis. The expression stability of the candidate reference genes between the tissues was evaluated by comparing the threshold values (Ct) and using the three commonly used statistical programmes geNorm, NormFinder and BestKeeper. Comparisons of the data obtained with the three statistical methods of calculation ranked RPS 8 as the best reference gene for normalisation of gene expression in olfactory-related tissues in *Bicyclus anynana*.

POSTER 2.

Adaptive significance of pre-overwinter copulation in females with non-overwinter males in the Japanese common grass yellow, *Eurema mandarina*

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Both sexes of autumn morph adults of the Japanese common grass yellow, *Eurema mandarina*, overwinters, while the summer morph ones do not. Such a wing polyphenism is regulated by either photoperiod or temperature throughout their larval stage. Because of different reaction norm between the sexes, the autumn morph adults coexist with the summer morph males in late autumn, during which autumn morph males show little mating activity. Consequently, mating between summer morph males and autumn morph females frequently occurred. Thereafter, mated autumn morph females re-mate with autumn morph males next spring. Two hypotheses for the adaptive significance of pre-overwinter mating by autumn morph females have been proposed. One is a nuptial gift hypothesis, and the other is a risk hedge hypothesis. To test these hypotheses, we examined the onset of oviposition after overwintering, compared to the re-mating frequency. Because the number of eggs loaded and the degree of the spermatophore decline in the bursa copulatrix inform the activity of oviposition and re-mating, wild autumn morph females were collected in November, late March, April and early May. Decrease in the number of eggs loaded in autumn morph females indicated that they started oviposition in the middle of April when the deciduous host plants began to sprout. The shape of the spermatophore suggested that autumn morph females could re-mate with autumn morph males after early April. Although the eupyrene sperm of the summer morph males remained in the spermatheca just after the overwinter, the nuptial gift hypothesis is more plausible than the risk hedge one, because most females re-mated in the spring before the onset of oviposition. Significantly longer eupyrene sperm of summer morph males than that of autumn morph males ones suggested a counter adaptation for female mating strategy, because of severe sperm competition with autumn morph males in the spring.

POSTER 3.

Biogeography of butterfly oviposition preference and larval performance on a native and invasive host plant

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Invasive and introduced species can have dramatic ecological consequences for native species and communities by altering community interactions and creating new patterns of selection on native species. In environments that have been recently invaded, formerly reliable host plant cues of phytogphagous insects may no longer be associated with adaptive outcomes for female oviposition and larval success. The invasion of *Alliaria petiolata* (garlic mustard) into the native habitat of *Pieris virginiensis* (West Virginia White butterfly) and its native host plant *Cardamine diphylla* (toothwort) in eastern North America is one such example. In New England, *P. virginiensis* females readily oviposit on the invasive *A. petiolata* plant, but the larvae are unable to grow and survive. The invasion of garlic mustard has expanded southward, where it is now present in *P. virginiensis* populations in West Virginia and Virginia but not in North Carolina. We are studying the ecological and evolutionary consequences of this invasion for southeastern populations of *P. virginiensis* using oviposition preference and larval feeding experiments with toothwort and garlic mustard. Our preliminary studies in North Carolina revealed wide variation among individual females in their oviposition preferences for the two hostplants. Hatching success did not differ between the two host plant species, but larval survival to 3rd instar was much lower on the invasive garlic mustard than on native toothwort. This suggests that the *A. petiolata* invasion could be a strong force driving selection for oviposition preference in adult *P. virginiensis* females.

POSTER 4.

Evolutionary aspects of lycaenid acoustic emissions

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Among insects acoustic signalling is a widespread form of communication, generally effective in defence, enemy threatening or mating. Even though the occurrence of this kind of communication between distantly related species is scarce, studies performed during the last 20 years suggest that the exchange of acoustic signals plays a fundamental role in butterfly-ant interaction. We recorded and analysed acoustic emissions produced by larvae and pupae belonging to 10 lycaenid species, chosen to cover the range of all possible degree of symbiotic associations (i.e. from "not myrmecophilous" to "obligate myrmecophilous"), as well as of their related ants. Every lycaenid species investigated, independently from the level of myrmecophilous association, showed the ability to produce highly specialised acoustic emissions which were carefully described and characterised for each group. Multivariate analyses were also performed to assess patterns of variations/similarities among individuals, species, phylogenetic and geographic distant sets or ant-association levels. Our results show that acoustics have multiple and sometime opposite functions according to the life cycle of the organism which emits the signal. The similarity of acoustic emissions produced by some lycaenid species and their associated ants reveals a role of acoustics in fostering myrmecophilous interactions. We discuss our findings in the light of evolutionary perspectives, trying to predict potential associations based on acoustic emission similarity and to elucidate the complex mechanisms responsible for the origin and the maintaining of ant-butterfly associations.

POSTER 5.

Targeting the genes involved in male sex pheromone reception in the butterfly *Bicyclus anynana*: a step forward for understanding the evolution of chemical communication.

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Olfactory communication using sex pheromones is important in mate choice, species recognition and sexual selection. The composition of the sex pheromone has been identified for hundreds of moth species, and Lepidoptera have thus become the main taxonomic group for studying the mechanistic basis, and the evolution, of sex pheromone communication. Stabilizing selection supposedly maintains the "lock-and-key" specificity of the interaction between sex pheromone components (the keys) on the one hand, and olfactory receptors and associated pheromone binding proteins (the locks) on the other hand. Yet, the huge diversity of chemicals forming the sex pheromone of closely related species in Lepidoptera, including the *Bicyclus* genus (Bacquet et al, in revision), leads us to question that stabilizing selection is the main selective force acting on the evolution of sex pheromone communication in Lepidoptera. Here we aim at identifying the genes involved in male sex pheromone reception in the model butterfly *B. anynana* and assess how sex pheromone reception evolved across closely related species of the *Bicyclus* genus. For this, we used the transcriptome recently produced for *B. anynana*

(Nieberding et al, in prep) and we targeted the transcripts overexpressed in both male and female antennae compared to adult brains. Using protein sequences (BLASTP), we selected 30 candidate genes that were related to known families involved in chemical reception of insects: olfactory receptors (comprising receptors to general odorants and pheromones), odorant binding proteins and chemosensory proteins. We validated the activity of the candidate genes using quantitative real time PCR assays that quantified antennal expression levels between groups of females reared in various olfactory environments and experiencing different reproductive histories.

POSTER 6.

Spatial distribution of fruit feeding butterflies along an altitudinal gradient in Brazil

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Understanding diversity patterns and the mechanisms underlying those patterns along elevation gradients is really important for conservation efforts in the ecosystem. The aim of this work is to determinate the distribution pattern of fruit feeding butterflies diversity (alfa and beta) and the biotic and abiotic mechanisms causing this pattern. The study was taken in Serra do Cipó, southeast Brazil. Serra do Cipó is located in the south of Espinhaço Range, a group of mountain that divide two hotspots in Brazil: Atlantic Forest from Cerrado. The climate is considered Cwb in Köppen classification. We had 7 elevations beginning from 800m to 1400m asl. Each elevation had three transects with four VSR traps with rotten banana as bait each. We collected in four different months during a year. In each elevation we measured temperature, humidity (as the abiotic factors), plant richness and abundance (as the biotic factors). We found 430 butterflies belonging to 51 species. In 800m there were 3 exclusive species: *Siderone marthesia*, *Hermeuptychia atalanta* and *Nica flavilla* and in 1400m there was only one *Fosterinaria pronophila*. Abundance (F18=6,98; p=0,024; R2=0,25) and richness (F18=15,628; p=0,0009; R2= 0,46) were smaller in the higher elevation, and beta did not differ along the gradient. The interaction between the temperature and humidity determines the distribution of richness and abundance of the butterflies, but the area, and biotic factors did not. In a global warming scenario, the distribution of butterflies can change and threat mostly species from the top hills, that usually are the most endangered species. Acknowledgement: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

POSTER 7.

Geographic mosaics of phenology, host preference, adult size and microhabitat choice predict butterfly resilience to climate warming

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The climate-sensitive butterfly *Euphydryas editha* exhibited interpopulation variation in egg placement and phenology. This should expose individuals to diverse thermal environments, which we investigated by measuring “eggspace” temperatures adjacent to natural egg clutches in populations distributed across a range of latitudes (36.8-44.6) and altitudes (213-3171 m). Eggs laid >50 cm above the ground averaged 3.1C cooler than ambient air at 1 m height, while those at <1 cm height averaged 15.5C hotter than ambient, ranging up to 47C. Because of differences in egg height, eggs at 3171 m elevation and 20.6C ambient air had mean eggspace temperatures 7C hotter than those at 213 m elevation and ambient 33.3C. Experimental eggs survived an hour’s exposure to 45C but were killed by 48C, suggesting that thermal stress occurs in nature. Interpopulation variation in phenology also strongly influenced thermal environment and buffered exposure to thermal stress. At sites with hotter July temperatures, the single annual flight/oviposition period was advanced such that eggs were laid on earlier dates, with cooler ambient temperatures. The insects possessed two mechanisms for advancing egg phenology; they could advance timing of larval diapause-breaking and/or shorten the life cycle by becoming smaller adults. The mean weight of newly-eclosed females varied among populations by more than a factor of two, suggesting that variable size did influence phenology. Possible options for in situ mitigation of thermal stress include further advancing phenology and raising egg height. We argue that these options still remain, as evidenced by current patterns of variation and by failure of *E. editha* to meet expected biogeographic relationships, such as the expectation that populations at equatorial and poleward range limits be restricted to higher and lower elevations, respectively. This optimistic example shows how complex local adaptation can generate resilience to climate warming.

POSTER 8.

Beyond offspring number-size trade-offs

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Nutritional stress, at levels experienced in the field, can affect butterfly fecundity, hence fitness. Such fitness effects may be magnified if egg composition also varies. In laboratory experiments, we fed adult female *Speyeria mormonia* 50% of the sugar-water diet imbibed by control females. In addition, we restricted the diet of last instar larval S. mormonia, such that experimental insects experienced periodic starvation while control insects had continuous food available. We measured age-specific fecundity, egg dry mass, egg protein, egg triglycerides and egg glycogen. We document decreases egg mass with age and show that egg triglycerides decrease in response to nutritional stress at either life stage. Changes in egg protein and glycogen, in the context of dry egg mass, are also reported. The results are placed in the context of the butterfly's life history.

POSTER 9.

Interactive effects of resource limitation and genetic background on the metabolic performance of the Glanville fritillary butterfly

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Metabolic performance in eukaryotes is largely determined by oxygen and energy availability, but it also varies with genetic background. One prominent example of a strong single-gene association is between metabolic performance and polymorphism in the gene Pgi, which encodes the glycolytic enzyme phosphoglucose isomerase. Whether performance is primarily limited by energy or oxygen availability, and how these limitations interact with genetic background, remain poorly understood. In the Glanville fritillary butterfly (*Melitaea cinxia*: Nymphalidae) individuals possessing the AC genotype in a SNP within the coding region of Pgi (Pgi_111) have significantly higher flight metabolic rate than the AA homozygotes. We measured the metabolic responses of the AA and AC genotypes under three resource manipulations: 1) Larvae reared under standard (28/15C) versus low-temperature (23/10C) conditions, 2) Adult butterflies maintained on a standard diet (20% sugar water solution) versus under starvation (water only), and 3) Adult butterflies flown under normoxic (21% O2, 79% N2) versus hypoxic (10% O2, 90% N2) conditions, during which flight metabolic rate was recorded. Metabolic response was measured through assaying four metabolites (glucose, trehalose, triglyceride and lactate) extracted from hemolymph. Here I will present results on how metabolism is affected by resource limitation, and how this response is influenced by the Pgi genotype.

POSTER 10.

Immature stages of *Kolana* (Lycaenidae), with a preliminary assessment of functional morphology and distribution of nectary organs in Neotropical Eumaeini

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The productions of reward secretions by specialized larval glands play a critical role in the maintenance of symbiotic interactions between butterflies and ants. In Eumaeini (Lycaenidae), the nectary organs consist of a single gland opening dorsally on the A7 abdominal segment, named dorsal nectary organ (DNO). Here, we describe through both light and scanning electron microscopy the immature stages of two Eumaeini species, *Kolana ergina* and *Kolana ligurina*. Moreover, we present a preliminary assessment of the external morphology, distribution, and functionality of DNO in Neotropical Eumaeini. Larvae of *Kolana* are not tended by ants; they have the body covered by large pairs of scoli-like structures, clusters of perforated cupola organs (PCOs), and completely lack DNOs. Pupae present conspicuous dark spots dorsally, that confer a face-like color pattern. The absence of nectary organs in *Kolana* contrasts with the pattern of the remaining Eumaeini, whose larvae always have DNO. Nectary glands may be classified in two types: (i) functional – with the opening surrounded by a large number of PCOs and dendritic setae, producing secretions when tended by ants, and (ii) non- functional – with few PCOs and dendritic setae, not producing visible secretions even after ant palpation. There is considerable variation in the occurrence of these types of DNO in different genera. For example, the sections Panthiades and Thereus have a prevalence of members with functional glands, while some sections like *Eumaeus*, *Atlides*, and *Brangas* have only members with reduced non-functional DNO. Variations in the functionality of DNO, however, may occur within the same genus, reflecting different life strategies and degrees of interaction with tending ants.

POSTER 11.

Specific host-ant associations reveal cryptic species in the myrmecophilous butterfly genus *Aricoris* (Riodinidae)

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The myrmecophilous butterfly genus *Aricoris* (Nymphidiini) comprises 24 species typically occurring in open and dry habitats in the neotropics. The taxonomy of this genus is based exclusively on adult morphology and the validity of some taxa has been debated for nearly a century. During studies about the life history of *Aricoris chilensis*, which includes five synonyms, we perceive the existence of a complex of cryptic species, with sympatric species associated with different host-ant species. Here, we review the '*chilensis* complex' based on ecological and morphological information of immature stages. Behavioral observations were taken in the mountainous region of Cordoba, Central Argentina. Using early-stage morphology and ecological data from symbiotic interactions between larvae, ants and host plant, we could solve some longstanding taxonomic problems. Larvae of *A. chilensis* are gregarious, fed on Asclepiadaceae, and are tended by *Camponotus punctulatus*, being *Aricoris susanae* a new synonym. Other *Aricoris* species preferably fed on *Acacia caven* (Fabaceae), but they differ as tending ants. *Aricoris cisandina* Reval. is obligately associated with *Camponotus mus*, while *Aricoris cosquinea* Reval. is tended by *Brachymyrmex*. Moreover, it was possible to identify a new species of *Aricoris* associated with a distinct *Camponotus* species. Tending ants of *Aricoris* vary greatly in morphology (size) and behavior (recruitment and aggressiveness). Morphological differences in larval ant-organs associated with distinct host-ant species are remarkable. For instance, *A. cosquinea* is constantly tended by dozens of tiny *Brachymyrmex* and has all functional ant-organs since hatching, while larvae of *A. cisandina* are tended by large workers of *C. mus* and have well developed tentacle nectary organs. These differences in immature stages make identification easier at this stage than in adults. Our results obtained for a small region of Argentina show that a correct understanding of the specific limits on *Aricoris* necessarily involves knowledge about their immature stages.

POSTER 12.

Nirodia belphegor (Lepidoptera: Riodinidae), life history and systematic position of an endangered butterfly from the "campos rupestres" of southeast Brazil

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Due to its continental extension and great environmental heterogeneity Brazil lacks basic biology knowledge for several butterfly species. Such information is critical to identify threatened species and outline conservation strategies. In this sense, the "National Action Plan for the Conservation of Lepidoptera" started in 2005, included the study of 58 threatened Lepidoptera species among its main actions. *Nirodia belphegor* (Riodinidae) is a critically endangered butterfly confined to the campos rupestres; a high-altitude rock outcrop vegetation from southeast Brazil. The aim of this study is to unveil the biology of *N. belphegor* and evaluate its systematic position. Adults were collected and legs were plucked from each individual and kept for molecular analysis. Total DNA was extracted, and the anterior part of mitochondrial COI gene was sequenced; a Bayesian analysis was conducted using *Baeotis* as outgroup and including related genera of Riodinini to establish its phylogenetic position (*Amarynthis*, *Chorinea*, *Panara*, *Lyropteryx*, *Necyria*, *Ancyluris*, and all species of *Rhetus*). *Nirodia* occurs exclusively above 1000 m, and adults were found resting upside down on rocks or feeding on flowers. Females searched for host plants during the hottest hours of the day, depositing 1-2 eggs on leaves of *Microstachys serrata* (Euphorbiaceae). The nonmyrmecophilous larvae underwent six instars. Larvae are covered with abundant setae. Early instars are reddish, becoming gray and cryptic in the last instars. Pupae are cryptic with several spines and projections. Phylogenetic analysis showed that *Nirodia* is part of *Rhetus*, suggesting that the generic status should be changed. Our data supports that *Nirodia* is the only species in the clade which is associated to high mountains, different from other congeneric that occurs in lowlands. The discovery of the biology and clarification on its systematic position are essential for the establishment of better and more effective conservation actions for this magnificent Brazilian butterfly.

POSTER 13.

The effects of mildew-infected diet on the butterfly *Melitaea cinxia*

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In the islands of Åland, Finland, ribwort plantain is a shared host plant to the Glanville fritillary butterfly (*Melitaea cinxia*) and the powdery mildew fungus *Podosphaera plantaginis*. Both *M. cinxia* and *P. plantaginis* persists as metapopulations in Åland and in some locations they co-exist. Recent studies have shown that *M. cinxia* larval development until diapause is affected by mildew-infected diet. Here, we studied the natural variation in tolerance to mildew-infected diet by using *M. cinxia* families from natural populations that during the last years have, or have not, experienced mildew infections. In addition we follow the development of butterflies until adulthood. We show that populations respond differently to mildew-infected diet and that the effects persist until after diapause but are gone when larvae pupate.

POSTER 14.

Seasonality maintains alternative life history phenotypes in butterflies

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Many butterflies express discrete alternative phenotypes (polyphenisms) in relation to predictable environmental variation. However, the evolution of alternative life history phenotypes remains poorly understood. Here, we analyze the evolution of alternative life histories in seasonal environments by using temperate butterflies as a model system. Temperate butterflies express alternative developmental pathways of diapause and direct development, the induction of a certain pathway affecting fitness through its life history correlates.

We develop a methodologically novel and holistic simulation model and optimize development time, growth rate, body size, reproductive effort and adult lifespan simultaneously in both developmental pathways. The model predicts that direct development should be associated with shorter development time (duration of growth) and adult life span, higher growth rate and reproductive effort, smaller body size as well as lower fecundity compared to the diapause pathway, because the two generations divide the available time unequally. These predictions are consistent with many empirical data. Our analysis shows that seasonality alone can explain the evolution of alternative life histories.

POSTER 15.

Insights into adaptive radiation of butterflies (Mycalesina) from species distributional modelling: Africa and Madagascar compared

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Species distributional modelling (SDM) is today widely used in fields like conservation biology and invasion biology. Building environmental niche models based largely on georeferenced museum specimens can provide testable insights into the shape and contiguity of species ranges, which environmental factors are most important in explaining the species range assuming environmental equilibrium, the potential for colonisation of new environments, and any relationship between environment and diversification. In the phylogeny of the satyrine butterfly subtribe Mycalesina, entire radiations or large clades are endemic to particular biogeographic realms, subjected to differing climatic and vegetational histories (particularly regarding the principal hostplants, Poaceae). It now looks like African and Madagascan radiations may differ fundamentally in that expansions have happened into grasslands and rainforests, respectively. Based on over 13000 Afrotropical point records and a standardised set of GIS surfaces for approximately year 2000 and at 1 km. resolution, models were built using MAXENT at hierarchical levels in the phylogeny from species upwards. Here, distributions and diversification patterns of sister taxa in the diverse radiations *Bicyclus* (about 95 species) and *Heteropsis* (about 70 species) in Africa and Madagascar are compared. The main question addressed is to what extent may climatic adaptation have driven the ecological speciation of these butterflies, or rather has diversification happened in the face of environmental niche conservatism of particular clades?

POSTER 16.

Historical and geographic variation in wing melanin an alpine butterfly in relation to climate

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Colias butterflies adapt to local climate conditions along latitudinal and elevation gradients through differences in hindwing melanin, allowing them to achieve the body temperatures needed for flight, mating and oviposition. The alpine areas of Colorado have seen greater increases in temperature over the past fifty years than other parts of the United States on the order of 0.4 C per decade. We used *Colias meadii*, a species restricted to subalpine and alpine habitats in the Rocky Mountains, to explore geographic variation and historical changes in wing melanin. We sampled museum specimens of *C. meadii* from across their geographic range for the last 120 years, focusing on past 50-60 years. We photographed each specimen to measure grey level as a metric of hind-wing melanin. By mapping the location of these data and relating grey level values to summer temperatures, our preliminary results suggest that melanin increases with elevation, but not with latitude, across the range of this species. Ongoing analyses will evaluate whether wing melanin has changed in association with climate changes over the past 50 years in the region.

POSTER 17.

Ants provide visual cues for oviposition in the facultative myrmecophilous butterfly *Parrhasius polibetes* (Lycaenidae)

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Selection of an adequate site for oviposition is important for lepidopterans since caterpillars have reduced mobility, and therefore are unable to choose the place where they will live. While searching for a site to lay their eggs, females may rely on visual, olfactory, gustative and tactile cues, which usually originate from the host plant. However, the presence of natural enemies or mutualistic partners can also be important for oviposition site selection in egg-laying butterflies. In the Brazilian cerrado savanna, larvae of the facultative myrmecophilous lycaenid, *Parrhasius polibetes*, feed on inflorescences of many species of plants, including the shrub *Schefflera vinosa* (Araliaceae), which frequently hosts aggregations of the ant-tended treehopper *Guayaquila xiphias* (Membracidae). Caterpillars growing near these aggregations benefit from increased survival, and females prefer to oviposit on inflorescences where they are present. Given the conspicuousness of ant-treehopper associations, we investigated whether gravid butterflies would use them as a visual cue for oviposition. Field experiments were carried out using paired branches of *S. vinosa* containing dried ants and/or treehoppers; butterfly eggs were counted and removed daily. Females preferred to oviposit on branches containing the visual sign of ants, whereas the visual sign of treehoppers alone did not trigger a significant response. Eggs were laid in greater numbers when the image of ants plus treehoppers was present. This reveals the importance of ants as visual cues for oviposition site selection in *P. polibetes*, and also enhances the relevance of the combined visual signal from ants and treehoppers for egg-laying decisions by females. The study of the cues used for oviposition by butterfly species with different levels of myrmecophily may reveal interesting patterns in host plant selection, especially in ant-rich environments such as the cerrado savanna. (FAPESP, CNPq).

POSTER 18.

Changes in predator community structure shifts the efficacy of two warning signals in Arctiid moths

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Polymorphism in warning coloration is puzzling because positive frequency- dependent selection by predators is expected to promote monomorphic warning signals in defended prey. We studied predation on the warning-coloured wood tiger moth (*Parasemia plantaginis*) by using artificial prey resembling white and yellow male colour morphs in five separate populations with different naturally occurring morph frequencies. We tested whether predation favours one of the colour morphs over the other and whether that is influenced either by local, natural colour morph frequencies or predator community composition. We found that yellow specimens were attacked less than white ones regardless of the local frequency of the morphs indicating frequency-independent selection, but predation did depend on predator community composition: yellows suffered less attacks when Paridae were abundant, whereas whites suffered less attacks when Prunellidae were abundant. Our results suggest that spatial heterogeneity in predator community composition can generate a geographical mosaic of selection facilitating the evolution of polymorphic warning signals. This is the first time this mechanism gains experimental support. Altogether, this study sheds light on the evolution of adaptive coloration in heterogeneous environments.

POSTER 19.

The band matters: Evidence for disruptive coloration in a butterfly - the Banded Peacock

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Many animals have evolved defensive coloration to reduce predation. Crypsis can consist of background matching or disruptive coloration—coloration that renders the outline of the animal difficult to detect and recognize as a prey item. Although much evidence supports background matching, very little exists for disruptive coloration. We used paper-plasticine models to test the hypothesis that the banded peacock butterfly, *Anartia fatima*, has evolved coloration that functions disruptively. We set out three model types: 1) natural, 2) banded margin (yellow band relocated to the wing margin), and 3) broken margin (yellow band broken into individual pattern elements along the margin). We placed 297 models of each type in disturbed tropical forest in central Panama and monitored attack rates on models over three days. Models exhibited an overall avian attack rate of 8.5%, with banded margin models attacked most, broken band models with intermediate predation, and natural models with the least predation. These results support the hypothesis that the natural band disrupts the butterfly's visual outline and thereby reduces predation. Moreover, if the band is broken into subsegments, there are unexpected increases in predation that were not predicted by conventional disruptive coloration theory. This finding suggests that the two pattern types of potentially disruptive coloration are not equally adaptive.

POSTER 20.

Revealing variation in a *Pieris oleracea* population adapting to invasive garlic mustard (*Alliaria petiolata*)

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The invasion of garlic mustard, *Alliaria petiolata*, into the range of *Pieris oleracea* has introduced this native North American butterfly to an evolutionary trap, whereby recognition of chemical oviposition cues causes female butterflies to oviposit on the invasive plant rather than the normal native host, at a cost to the development and survival of their larval offspring. Negative fitness consequences for the native butterfly set the stage for adaptation by natural selection: in an area of sustained co-occurrence, *P. oleracea* may be escaping this evolutionary trap. In this population, research has demonstrated a wide range of larval performance and survival that correlated positively with female preference for garlic mustard, a correlation not seen in naïve populations. Our primary objectives were to establish a genetic basis for highly variable growth and survival of *P. oleracea* on a garlic mustard diet, and to assess whether the ability to successfully use garlic mustard as a larval host has increased with intense and sustained exposure to the invasive plant. We compared larval success on garlic mustard among families of half-sibs (as a proxy for genetic variation within populations) from Lenox, MA, where garlic mustard is well established (GM+), and families from Texas Falls, VT, where garlic mustard had not yet invaded (GM–). We found substantial diet- dependent variation in growth and survivorship among families from both sites, confirming that heritable variation is present and accessible to selection pressures. We discovered larval success on garlic mustard in the GM- population, which suggests larval ability to perform well on garlic mustard may represent a remnant genetic polymorphism and is not unique to the Lenox (GM+) population.

POSTER 21.

Dissecting geographic variation in diapause phenology of the orange tip butterfly

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Variation in environmental suitability for growth and reproduction throughout the year at temperate latitudes has given rise to intricate adaptations in the timing of life history events of flora and fauna. The temporal aspect of life cycle traits is important for fitness, as it affects the biotic and abiotic environment an individual encounters. These phenological traits can vary geographically because of local adaptation as well as via phenotypic plasticity. The degree to which genes and environments, and their interaction, contribute to the geographic phenological pattern influences how the species or populations respond to short-term environmental fluctuations and long- term ecological changes. We have looked at two life cycle characteristics that affect spring phenology in the orange tip butterfly, Anthocharis cardamines; diapause duration and post-diapause development. We found local adaptation in various components of post-diapause development as well as in diapause duration. We hypothesise that these adaptations reflect local host plant phenology as well as local seasonal progression and spring temperature profiles. These results emphasise the importance of considering local ecological conditions when studying adaptations across geographical gradients, such as latitude.

POSTER 22.

Mimetic resemblance of butterflies from an avian visual perspective

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Various aspects of mimicry such as frequency dependent selection, predator learning and mimetic perfection have been fairly well studied in the past. However, mimetic resemblance has largely been quantified in the past based on human visual assessment. Since birds which exert arguably the strongest selection on the evolution of butterfly mimetic resemblance have visual capabilities that differ considerably from human vision, mimicry needs to be assessed through their visual perception. Here we investigate mimetic resemblance from an avian visual perspective using Indian butterfly mimicry rings. Reflectance spectrometry sensitive to the entire bird-visible spectrum (300-700 nm) provided an objective measurement of wing colors of butterfly models and mimics. Visual modelling was then used to determine whether wing colors of models and mimics were discriminable from an avian visual perspective. Specifically, we predicted less mimetic perfection in sex-limited mimics (when only one sex mimics and the other sex has non-mimetic ancestral wing colors) because sex-specific selection on wing colors may be hampered by genetic correlations between mimetic and non-mimetic sexes. Contrary to this expectation, we found that mimetic sexes of sex-limited mimics were as good mimics as sexually monomorphic mimics. This suggests that mimicry exerts very strong selection on wing patterns, and to achieve mimetic perfection, this selection has broken down any genetic correlations that existed between mimetic and non-mimetic wing patterns. However, we found that in some species males had more intense colors compared to females, indicating the sexes may show subtly different wing colors in response to either sexual selection on male ornaments or natural selection on females to be more protectively patterned. We discuss the significance of these findings with respect to the evolution of butterfly wing colors under natural and sexual selection pressures.

POSTER 23.

Honest signal of chemical defence is costly for the aposematic Wood tiger moth *Parasemia plantaginis*

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Maintenance of colour polymorphism in aposematic organisms requires evolutionary explanation because selection by predators is expected to wipe out less efficient variants of warning signals. In Europe, wood tiger moths *Parasemia plantaginis* have two distinct male colour morphs, yellow and white. Wing pattern of the yellow morph has been shown to be more efficient a warning signal against avian predators but the two morphs still co-exist. When threatened, the moths produce defensive secretions from their abdomen and/or from their thoracic glands. We tested whether the two male morphs have different anti-predator strategies that result in equal protection: investment either in an efficient warning signal (yellow) or in efficient chemical defence (white). We reared moths in standardized laboratory conditions and measured the amount of thoracic and abdominal defensive secretion from both morphs. We also tested the efficacy of the abdominal and thoracic defence secretions by using their natural predators, and measured the life history costs of defence. Contrary to our expectations, we found that the defensive secretions of the yellow morph were better both in quantity and quality, but it also incurred higher costs. Forced production of defensive secretion decreased the reproductive output of both colour morphs but shortened the life-span of the yellow morph only. Our result suggest that the more conspicuous yellow warning coloration functions as an honest signal of defence but the yellow morph also pays the higher cost from its antipredator strategy which may offer an explanation for the coexistence of two male morphs in this species.

POSTER 24.

Sex difference in development time: a universal prerequisite for sexual size dimorphism in insects (with the exception of butterflies)

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The degree and direction of sexual size dimorphism (SSD) vary greatly among insect species. There are just three principal, non-exclusive possibilities how sexual differences in body size may arise during the ontogeny. The individuals of the larger sex may either (1) be larger from the very beginning, (2) grow faster or (3) grow for a longer time. Nevertheless, empirical evidence concerning proximate causation of SSD is scattered, and the data used to infer ontogenetic determinants of SSD have not always been appropriate for this purpose. I use a comprehensive literature-derived database to address the significance of sex differences in larval development time (SDTD) as a proximate source of SSD in insects in general, and butterflies in particular. In a clear majority of insect species (79% of 169 species), SSD and SDTD were qualitatively congruent, i.e. the larger sex had also a longer larval development. In strongly size-dimorphic species, the qualitative correspondence between SSD and SDTD was nearly universal. Consistently, in a phylogenetically diverse array of insect clades, SDTD increased with increasing SSD across species. The results indicate that the evolution and maintenance of high SSD values are rarely possible without a prolonged development of the larger sex. Butterflies seem to form a remarkable exception here, showing a weak or non-existent among-species correlation between SSD and SDTD. Sex differences in growth rate thus likely play a more prominent role in the determination of SSD in butterflies. To which extent growth rate mediates SSD in insects more generally requires further studies which should ideally be based on detailed monitoring of larval growth schedules. The increase in SDTD with increasing SSD is consistent with the idea that the widespread phenomenon of protandry (the emergence of male adults before females) may primarily be an incidental by-product of sex-divergent selection on body sizes.

POSTER SESSION: Genomic basis of adaptations

POSTER 25.

Speciation genomics in *Leptidea* butterflies

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Understanding the key forces underlying population differentiation and speciation, the forces that ultimately generate biodiversity, is a fundamental quest in evolutionary biology. To address this we work with three closely related cryptic species of the wood white *Leptidea* butterflies. Understanding the genetic basis of reproductive isolation is of particular interest in this system since the species are cryptic and the traits involved in mate preference and species identification likely are related to behavior and chemical signaling, traits that have been largely overlooked in previous speciation research. The three species split relatively recently and provide a good system for studying genomic causes and consequences of local adaptation and early differentiation processes. We know from previous research that specific regions of the genome may act as drivers of differentiation. By using a combination of classical genetic and novel genomic tools we will try to identify and characterize the genetic regions that might have been involved in the build-up of reproductive isolation between these cryptic species of butterflies. In addition, within one of the species there is considerable variation in karyotype structure among populations from different areas of the distribution range and we will investigate the role of rapid karyotype evolution on the recombination landscape and how chromosome rearrangements can affect genome evolution and population differentiation processes.

POSTER 26.

Genome-wide analysis of immune genes and their local adaptations in *Pieris napi*

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Different environments pose different immunological threats, and therefore a population will adapt their immune system accordingly, making immunology an interesting study frame for local adaptations. The Green Veined White (*Pieris napi*) is highly suitable species for identifying local adaptation in immunity. They are common and widespread with limited gene flow between populations; have a well-defined ecology, a short generation time, and are easy to rear in the lab. By using annotated genomes of other insects I am identifying orthologs of immune genes in the *P. napi* genome, in combination with RNA-Seq on infected individuals. Using population re- sequencing data, within and between population polymorphisms in these immune genes are then being identified and characterized.

POSTER 27.

Using population re-sequencing data and a poor genome to scan for selection

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What genes are involved in local adaptation? How does genetic variation affect life history traits? Questions such as these fascinate our research community, but are hard to answer in novel species in an economical and timely fashion without a high quality genome. Although advances in sequencing technologies make it possible to sequence whole genomes in days, effectively assembling and using such data is challenging. We have developed a method called MESPA (Mining Exons for Scaffolding Poor Assemblies) that takes as input a candidate protein gene set along with a low quality genome and returns high quality gene models. Using MESPA in combination with Pool- Seq, I report on the genetic variation likely involved in local adaptation and species differences across a range of butterfly species without having had to invest time and money in a high-quality genome.

POSTER 28.

Developmental signature of the ageing-related transcriptional profile in a seasonal butterfly

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Ageing is a by-product of natural selection shaping the life history of organisms to ensure maximal reproductive output in balance with optimal lifespan. To understand the public, evolutionarily shared mechanisms of ageing, it is crucial to understand the genetic regulation of lifespan in relation to adaptation in the relevant evolutionary environment. We use the butterfly *Bicyclus anynana* to study transcriptional patterns associated with seasonal developmental plasticity of adult life history. In response to seasonal temperatures during development, larvae develop into either fast reproducing but relatively short-lived wet season adults, or long-lived dry season adults that delay reproduction. The plasticity in life histories is assumed to be regulated by alternative genetic programs, activated by environmental cues. Using custom-designed microarrays, we probed the transcriptional profile of young and old butterflies developed in dry or wet season conditions, and observed substantial ageing-related expression changes. Approximately half of all gene expression changes were sex-specific, with females up-regulating stress response genes and down-regulating reproduction-related genes with age. In dry season adults, age-related expression changes were abrogated compared to the wet season morph. In particular, they lacked the age-related up-regulation of immune genes and the down-regulation of reproduction genes that we observed in wet season butterflies, likely contributing to their long-lived phenotype. Only a small number of genes showed seasonal expression bias independent of age, with several of these seasonally imprinted genes being related to Insulin signalling. The redeployment of this highly conserved nutrient-sensing pathway in the specific ecological circumstances of *B. anynana* illustrates the versatility of hormonal systems that can play additional roles in different life stages or environments.

POSTER 29.

Exploring the genetic and metabolic basis for the Alba phenotype in *Colias* butterflies, and its evolutionary implications.

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Colors and patterns in nature often have known adaptive value and are considered a visual representation of the selection process; therefore they provide important evolutionary insight. Butterflies in the genus *Colias* exhibit color variation both among, but more interestingly, within species. A color dimorphism, called Alba exists in females of many *Colias* species. This dimorphism causes a change in the wing ground color, from yellow-orange to white. The Alba allele is known to affect female physiology, behavior, fecundity, and overall fitness. Therefore, it is an excellent model for ecological genomic research and its study can provide evolutionary insight into the role of color variation within and among species. Unfortunately, the genetic and molecular mechanisms that control this color variation remain unknown. Here I will report on the results of our latest efforts using a range of genomic tools to identify the genetic and physiological basis of Alba. Our analysis includes RNA-Seq, QTL mapping, whole genome sequencing and Pool-Seq analyses.

POSTER SESSION: Dispersal and conservation

POSTER 30.

Population genetics of reintroduced large blue butterflies in the UK, and their source population

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The reintroduction of the large blue butterfly (*Maculinea arion*) into the U.K. following its extinction in 1979 has been one of the greatest success stories of invertebrate conservation. The reintroduced populations have both increased in numbers and colonized additional sites, and now represent the highest densities of large blues recorded globally. However, the number of individuals used for reintroduction were necessarily limited, and recent population genetic studies of the source population used, from Öland, Sweden, have revealed rather low genetic diversity and small effective population sizes. Introduced populations are initially small, and thus vulnerable to genetic drift and stochastic events. The level of genetic diversity maintained is important for the long-term persistence of populations and their evolutionary potential to react to, for example, climate change. Hence, we investigated the genetic diversity and differentiation of populations of the large blue nineteen generations after translocation on five sites in the UK, and seven sites on Öland, including the source population. We found similar levels of genetic diversity in the reintroduced and source populations, but the UK and Swedish populations were genetically differentiated; we also found significant genetic differentiation among reintroduced UK populations only a few kilometres apart. The reintroduced populations had several private alleles not found in the source population in 2011, and thus may already represent a unique subset of genetic diversity of the north-western populations of *M. arion*. This pattern of genetic differentiation is probably a result of the unique life cycle and dispersal patterns of the Large Blue. Our results show that the IUCN and other protocols followed in the 1990s for translocating and maintaining the maximum available genetic diversity during reintroductions were largely adequate for this species, and hence will be valuable for informing the growing use of reintroductions as a strategy for the conservation of endangered butterflies.

POSTER 31.

Evaluation of the influence of habitat conversion in the distribution of the endangered swallowtail butterfly *Euryades corethrus* (Papilionidae, Troidini) using Species Distribution Models (SDMs) and functional connectivity tools from GIS

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Land cover and soil usage affect Lepidoptera occurrence since the presence of the plants dictates the presence of the animals. Metapopulation models assume that the landscape is a mosaic of suitable and unsuitable habitats, with their spatial configuration and composition being determinant to dispersion. Thus, large agriculture areas can affect the environmental matrix, decreasing ecological connectivity and increasing the cost associated with dispersion and foraging of butterflies. In this study we use Species Distribution Models (SDMs) and functional connectivity tools from Geographic Information Systems (GIS) to evaluate the influence of agriculture in habitat loss of the endangered swallowtail butterfly *Euryades corethrus*. We produce SDMs for *E. corethrus* using occurrence data from inventories, online databases (GBIF, SPLink and JSTOR) and entomological collections, and current climate data from WorldClim. Environmental variable selection was performed by PCA and SDMs produced with five different algorithms (Bioclim, GARP, Maxent, SVM, Environmental Distance) in OpenModeller 1.4. Resulting SDMs were validated with AUC and TSS, adjusted to the minimum training presence and ensemble in a consensus model. Land cover data from GLC2000 was used to clip the fundamental niche distribution of *E. corethrus* regarding present day remaining suitable areas (i.e. native grasslands and non- managed livestock) aiming to outline a conservation strategy and conservation areas for the species. The analysis revealed that more than 70% of original distribution area was lost by habitat conversion into monocultures. Additionally, the remaining suitable areas were very fragmented, producing low quality landscapes. The GIS tools revealed that the functional connectivity of suitable areas affects the costs to foraging butterflies and viability of the local population thus altering metapopulation structure. We hope this improves conservation strategies for *E. corethrus* by defining adequate environmental configurations to enable successful foraging and dispersion.

POSTER 32.

Potential Distribution of *Stegosatyrus periphas* Godart, [1824] (Nymphalidae, Satyrini, Euptychiina) and factors influencing their distribution

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Stegosatyrus periphas Godart, [1824] (Nymphalidae, Satyrini, Euptychiina) is widely distributed in south Brazil, Uruguay and north Argentina, from sea level to 1,400 m. There is limited information about the factors that limit the distribution of this species. The aim of the present study is to determine potential environments and factors that may be determinant for the distribution of S. periphas using the species potential distribution modeling approach. From the 66 spatially uniques occurrence records, we used 47 points to train (generate) the models and 19 to test their predictive capability, with ten replicates. PCA and Mantel tests were performed to identify and remove the most correlated variables and keep those which could add high quality information. From the 19 environmental variables from Wordclim plus (digital elevation model) and slope from USGS HydroSHEDS (resampled to 0.0416 arc minutes (˜5km pixel)), eight were used to construct the model; a combination of slope, elevation and variations of temperature ranges and mean precipitation. Annual Precipitation and Precipitation of Wettest Quarter presented the most valuable information using Maxent, SVM and GARP algorithms. The models were cut off by ROC threshold and summed up. Due to mathematical differences between outputs we showed the model as a categorical map ranging from 1 to 3. The average AUC test and TSS were 0.92 and 0.87 respectively, indicating a good performance for the model. Grassland areas in south

Brazil and Uruguay (Pampa) seem crucial for the species. The Pampa is one of the most threatened biome at South America. Urgent efforts should be target for its conservation. Much benefit would accrue not only for *S. periphas*, but also for several other, possibly endangered, species.

POSTER 33.

Life history traits of butterfly assemblages reflect topography and landscape context of Czech nature reserves

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Establishment of nature reserves represents traditional and widely used approach to biodiversity conservation. In the Czech Republic, besides of National Parks and Landscape Protected Areas, over 2000 smaller-sized reserves exist, of which about 200 enjoy "national" status, being regarded as the most valuable conservation localities. In 125 of these national reserves (area range: 0.3 – 2030 ha), butterfly surveys had been performed, following a standard protocol (5 visits per year, timed survey method). We analysed the resulting data with regard to area, topography, borders geometry and habitats heterogeneity within the reserves and in their environs, using data on butterfly life history traits to explain differences in species composition and controlling for phylogeny. Butterfly assemblages composition was mostly influenced by reserves latitude, longitude, altitude, and prevailing habitats. After statistically controlling for these effects, the assemblages composition was still signifficantly affected by habitat heterogeneity within the reserves, and in their 2000 m environs. Larger reserves located in more heterogeneous landscapes hosted more specialists, whereas generalists tended to prevail in smaller reserves within homogeneous landscapes. Heterogeneous landscapes around reserves likely provide for easier dispersal, or population persistence, of more specialised species. Our results neatly illustrate that for butterflies, general conservation rules apply independently on prevailing type of protected habitat (grasslands, woodlands, wetlands).

POSTER 34.

Disturbance and climate interaction effects on a forest butterfly community in Vietnam

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Tropical forest species face multiple threats to their persistence including climate change, disturbance, habitat loss and fragmentation. Though butterflies have been critical indicators of how anthropogenic changes impact ecosystems globally, studies have been slow to develop in the tropics despite the fact that tropical forests are the centers of Lepidoptera biodiversity. Using a 10 year long-term (2003-2013) study of butterflies in Tam Dao National Park in Vietnam we examined the interaction of habitat diversity and climate on butterfly species in the context of environmental change (disturbance). Butterfly community changes over time were mixed across habitats but we found a pronounced decrease in forest-associated butterflies (especially *Ragadia crisilda* – 95% decline) in the closed-canopy forest transect in response to a large road having been built at that location. Local temperatures varied across the six transects/habitats with forests being generally cooler than other habitat types. We also found effects of disturbance (road construction) and cloudiness on local temperatures. Annually, we found a significant and negative association between temperature and forest-associated species abundance within forest transects. However, temperature had no perceptible impact on ruderal species abundance within any of the habitat types. This study emphasizes the importance of long-term monitoring and highlights the complex interactions between climate, habitat and disturbance which together and in combination can have significant effects on tropical butterfly community compositions.

POSTER 35.

Movement and functional connectivity in a guild of tropical fruit-feeding butterflies

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The consequences of habitat loss and fragmentation are well known for a variety of species, generally leading to population losses and reduced connectivity. In tropical habitats, forest fragments are surrounded by a variety of habitat matrices with unknown effects on the local butterfly fauna. In order to understand how fruit feeding butterflies move in such an anthropogenic landscape we conducted a mark-recapture study of butterflies trapped in three adjacent habitats: a forest fragment and two neighboring plantations that differed in structural complexity, one being more open and hotter than the other (coconut plantation vs *Acacia mange* plantation) . Butterfly movement was estimated from recaptured individuals. We collected data on 31 fruit feeding species, but analysed the ten most common ones. Data were collected for a year. We trapped 4575 individuals, varying from 1242 to 1739 in each habitat. Butterfly habitat preference correlated broadly with movement. Forest specialists tended not to venture into the more open planted forest and tended to fly into the *Acacia* grove. Generalists were less tied to a particular habitat, although we noticed differences between generalist in types of movement. We provide novel results for butterfly movement in tropical realms and discuss the implication of our study for habitat

management and restoration in the tropics.

POSTER 36.

Community structure and vertical stratification of fruit-feeding butterflies in two different landscapes of Atlantic Rain Forest

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The Atlantic Rain Forest has been reduced to only 8% of its original area and has a high level of threat due to the continuous and accelerated conversion of natural areas to anthropic systems. Fruit-feeding butterflies are excellent organisms for habitat monitoring diagnostic, since they present short life cycles, have well known taxonomy, and are easy to sample. The present study was designed to provide basic knowledge of fruit-feeding butterflies from the study site. Fieldwork was carried out during January to March 2014 in two distinct areas of Atlantic Forest in the state of São Paulo, Southeastern Brazil: a forest fragment and a continuous forest. Thirty-two traps baited with a mixture of banana and fermented sugarcane juice were distributed in two strata (canopy and understory) from edge to interior in the two areas. During four months, 799 individuals in 59 species have been sampled. The rarefaction analysis showed that the continuous forest was richer than the forest fragment, and species composition was highly different between the fragment and the continuous forest. Butterfly abundance was higher in the edge, decreasing towards the interior. The continuation of this study will surely improve the knowledge about fruit-feeding butterfly assemblages in the Atlantic Forest, and will provide subsidies for management and conservation of forest remnants in this important and threatened biome.

POSTER 37.

Population biology of *Memphis appias* (Hübner, [1825]) (Nymphalidae: Charaxinae) in Southeastern Brazil

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Seasonal climatic variations can lead organisms to vary in abundance and in their traits. *Memphis appias* (Hübner, [1825]) (Nymphalidae: Charaxinae) has been recorded abundantly in a forested area, enabling the description of basic population parameters and traits throughout time. Fieldwork was carried out monthly along two years, in a seasonal Atlantic Forest site in Southeastern Brazil, with five sets of ten baited traps alternating between canopy and understory. We recorded 1007 individuals of *M. appias*, 613 males (61%) and 394 females (39%). Monthly variation in number of adults was similar in both sexes, with marked peaks in September-October. The average forewing length of females (mean=3.173, SE=0.017, n=324) was greater than that of males (mean=2.915, SE=0.020, n=507; t=10.087, $p<0.001$). Mean forewing length decreased in the cold-dry season and increased during the warm-wet season for both sexes. Mean precipitation and humidity inside forest were good predictors of total number of individuals and size of males. Humidity in the previous month better explained variation in total abundance (R=0.615, $p<0.001$) and also male forewing length (R=0.577, $p<0.001$). Sex ratio was significantly different between months (G=44.293, df=24, p=0.007), with prevalence of males in most months. All recaptures (6%) but one occurred in the same sampling occasion, indicating replacement of adults in population in one-month period. Most recaptures occurred in the same stratum (90%), mainly in canopy, and vertical movement was predominantly upward (5 out of 6 events). Transect change was rare (3 out of 60 events) and reported only in females. Trap shift was more frequent in females (X=9.524, df=1, p=0.002), suggesting higher dispersion ability in this sex. Recapture data suggest that adults have restricted home ranges, and their robust thorax and vigorous flight may reflect other behavioral traits than dispersion, such as escaping from predators.

POSTER 38.

Temporal variation of fruit-feeding butterflies in the Atlantic Forest

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Long term studies are needed to describe accurately seasonal patterns of species diversity. Due to ease of sampling and the advanced taxonomic knowledge for identification of species, fruit-feeding butterflies can be considered good model organisms to characterize tropical insect diversity. The present study describes and compares two years variation in abundance and species richness of a fruit- feeding butterfly community in a seasonal Atlantic Forest site in Southeastern Brazil with two marked seasons: warm-wet from October to March, and cold-dry from April to September. Five sets of ten baited traps were distributed in five transects, alternating between canopy and understory. Samplings were done from October 2011 to September 2013. We recorded 3251 individuals of 80 species, representing the Nymphalidae subfamilies: Biblidinae (13.7% of total individuals), Charaxinae (50.9%), Nymphalinae (2.5%) and Satyrinae (32.9%) – tribes Brassolini (6.7%), Morphini (4.4%) and Satyrini (21.8%). Species richness and abundance fluctuations were similar for both years. The richness presented biannual pattern, one peak in February-April and another in September-October. Circular analyses showed

a non-random distribution of the total assemblage, which presented one peak of abundance in September-October. Subfamilies showed distinct patterns: Charaxinae (r1=0.61, r2=0.34) was markedly concentrated in the end of cold season in both years, concentration of Biblidinae (r1=0.10, r2=0.37) and Nymphalinae (r1=0.52, r2=0.20) were recorded in only one year. Satyrinae (r1=0.01, r2=0.10) had no clear peak of abundance, but its tribes separately had distinct concentration periods. Species composition was distinct between seasons (ANOSIM, R=0.207, p<0.001) but not between years (R=0.048, p=0.130). The periods of distinct abundance and richness of butterflies coincided with transitions between wet and dry seasons, during which foraging and reproductive activities may be facilitated. Overall, fruit-feeding butterflies were unevenly distributed throughout time in Serra do Japi, indicating that specific periods of the year can be considered more suitable for rapid surveys.

POSTER 39.

Dispersal abilities and habitat preferences of the *Euphydryas aurinia* complex

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Euphydryas aurinia complex consists of extremely variable populations across its distributional range and at least 3 subspecies occur in Italy showing slight morphological differences but major eco-ethological distinct features. We investigated the migration patterns of *E. a. glacegienita* inhabiting a site in the NW Alps (2000-2300 m) and of *E. a. provincialis* occurring in Mediterranean dry grasslands (700 m) using mark-release-recapture methods. We applied the Virtual Migration Model (Hanski et al. 2000) to estimate the parameters of emigration, immigration and mortality separately for males and females. We used survival and dispersal parameters to explore intra- and inter-population characteristics and investigated if those are intrinsic to each butterfly "entity" or vary with respect to habitat features (e.g. matrix properties) or density of conspecifics, nectar source and host plant. Our results showed that populations and sexes were markedly different in their dispersal patterns and survival parameters. *E. a. provincialis* individuals tended to move more frequently from patches and to cover longer dispersal distances than *E. a. glaciengenita* butterflies; males showed higher dispersal rates than females in both populations. The patch area had a substantial effect in both emigration and immigration rates for the Alpine population but not in the Mediterranean one. Mortality within patches was higher for *E. a. glaciegenita* (about 15%) whereas in the case of *E. a. provincialis* population most of mortality was observed during migration and was strongly influenced by the connectivity of the patch system. According to our data and to previous works, dispersal ability is not only a fixed species-specific trait, but is also highly sensitive to various selective pressures and can vary among populations of the same species, between sexes, habitat (isolated vs connected), and altitude (low altitude vs alpine level). Reference: Hanski I., Alho J. & Moilanen A. (2000) Ecology 81: 239-251

POSTER 40.

Sampling scheme optimization for microsatellite-based population genetic studies

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Genetic studies are more and more used in conservation and landscape ecology contexts. While the costs and time involved in sampling, laboratory work and analyses increase with the number of samples, the gain of accuracy of genetic estimates does not. Optimizing the sampling scheme is therefore important to ensure reliable estimation of genetic parameters at a minimum cost. We conducted a literature review in the Scopus database using "butterfl* AND microsatellit*" as keywords to investigate how sampling scheme of microsatellite-based population genetic analyses was usually done, focusing on three main aspects of the sampled butterflies: their number, the sex ratio and the period of sampling. From the 124 papers returned, 33 matched the required context: genetic diversity estimation and differentiation between populations. The number of butterflies sampled per population varied tremendously, from 1 to 100 (mean STD: 22.46 13.89). The sex ratio was explicitly mentioned in only 2 papers, both male biased. The period of sampling during the flight season was never mentioned; furthermore many studies pooled samples collected over several years (generally 2 consecutive years). We sampled *Boloria aquilonaris*, a bog-dwelling butterfly, in Belgium (9 populations, 70 individuals) and used microsatellite markers to estimate some genetic measures (observed heterozygosity, within population heterozygote deficit, genetic differentiation between populations and number of migrant per generation). We then conducted sensitivity analyses on these measures via downsampling of the data. Results confirmed how the sample size affects the estimate of genetic measures, already shown by previous studies; but, more importantly, they indicated that the sex ratio and the period of sampling can profoundly affect them too, potentially leading to different conclusions and consequently impacting conservation measures issued from these genetic studies.

POSTER 41.

Intraspecific variation and genetic structure of the Corsican swallowtail, *Papilio hospiton* Gené (Papilionidae)

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The Corsican swallowtail, *Papilio hospiton* Gené, is endemic to the islands of Corsica and Sardinia. Foodplants are restricted to a few Apiaceae and Rutaceae species. Natural hybrids between *P. hospiton* and *Papilio machaon* L., a widely distributed Palaearctic species, are frequent and partially fertile. Habitat destruction, disappearance of foodplants and changes in land usage are principal threats to this species. Formerly included in the IUCN Red List (1973) as one of most threatened butterflies, the status of *P. hospiton* has been dropped to a least concern taxon after studies made on Corsican populations (1996). At present, *P. hospiton* is listed as priority species in the EC Habitats Directives. The lack of updated information on the status of *P. hospiton* prompted us to start a multidisciplinary project at the ecological, genetic, and physiological levels. We present here preliminary data on the genetic characterization of *P. hospiton* based on the analysis of four mtDNA markers. Samples, including *P. machaon* and hybrid specimens, were captured in different areas of Sardinia. Corsican material came from pinned adults collected before 1996. A COII haplotype, already listed in GenBank, was shared by all Sardinian and Corsican specimens. Previously undescribed haplotypes were detected for COI, ND1 and ND5. Intraspecific variation was low: haplotype diversity ranged from 0.233 (COI) to 0.292 (ND5) and 0.318 (ND1). Nucleotide diversity from 0.0004 to 0.0008 with only few synonymous substitutions. Haplotype distribution across space was not uniform but overall divergence between the two islands, which shared the most common haplotypes, was not significant ($P>0.05$), reflecting the high vagility of the species. However, the finding of a unique haplotype in a few specimens from central Corsica would suggest the presence of a distinct sub-population. In hybrid specimens, presence of either *P.machaon* or *P.hospiton* haplotypes confirmed that hybridization can take place in both directions.

POSTER 42.

Assisted colonization of the endangered butterfly *Parnassius apollo* in southern Finland

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Assisted colonization (translocation of individuals across a barrier which hampers natural colonization) is a potentially important, but debated, conservation tool for a variety of organisms in a world altered by anthropogenic influences. Empirical research documenting how such a scheme could be achieved, or why it fails, is currently needed in order to improve our understanding of this conservation tool. *Parnassius apollo* is an endangered butterfly whose distribution has retracted dramatically during the 1900s across Europe. In Finland, *P. apollo* currently occupies only a fraction of the range of its suitable habitat and is apparently unable to (re-)colonize this habitat. Based on eggs collected from wild-caught females from the species' current Finnish stronghold, a population was reared in order to introduce larvae in an unoccupied, but highly suitable part of the Finnish archipelago. In 2009, a restrictive number of larvae (1 larva / 10 host plants) were introduced on 25 islands in the inner, middle and outer archipelago zones. In 2010, 9 islands situated in all 3 archipelago zones were (re)stocked with a high density of larvae (1/host plant). In 2011, *P. apollo* larval populations were found only on islands in the outer archipelago zone, which were then restocked. The species remained present here in the following years (2012, 2013) and is hence able to sustain a viable population unaided. Our findings demonstrate that putative suitable habitat may in reality consist of only a few sites where population establishment is possible. Hence, starting the introduction in many sites but then "zooming in" on a small set of promising sites was key to the success of this translocation.

POSTER 43.

Atlantic Forest butterflies in southeastern Brazil: Updating on endangered butterflies distribution, systematics and biology after three years of maximized inventories

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The research project "Atlantic Forest butterflies in southeastern Brazil" had been developed between 2011-2014 aiming to (1) identify and sample poorly known areas in different Atlantic Forest habitats, (2) make available and update data on butterfly ecology and conservation, (3) increase knowledge about most threatened species in Brazil. After three years our group achieved most of the main goals of the project, namely (1) field expeditions to make rapid and optimized inventories in sites with incomplete or poorly species lists to search for threatened butterflies in several different habitats in a wide region in the Atlantic Forest domain, (2) increasing knowledge updating data on butterflies distribution and occurrence, and (3) publication of inedited information. Concerning threatened butterfly taxa, the main results includes: (1) rediscovery of *Charonias theano* (Pieridae) in São Paulo State, as well as the distribution extension and confirmation of its presence in a protected area; (2) systematic position and distribution of the critically endangered *Euptychia boulleti* (Satyrinae); (3) new distributional data for four endangered nymphalid butterflies: *Actinote quadra* (Acraeini), *Episcada vitrea*, *Tithorea harmonia caissara* (Ithomiini) and *Paulogramma hydarnis* (Biblidinae); (4) description of the immature stages of the critically endangered *Actinote zikani* (Acraeini); (5) detailed descriptions of basic population parameters of two threatened swallowtails: *Parides burchellanus* and *Parides ascanius*, (6) ecological genetics of *P. ascanius* in coastal Rio de Janeiro, (7) rediscovery of *Morpho epistrophus nikolajewna* (Morphini) in a highly urbanized forest fragment in northeast Brazil. The future of the Brazilian threatened butterflies will depend on a combination of actions: the discovery and preservation of other populations; establishment of additional protected habitats, and management of captive butterflies for reestablishment of viable populations in the field. Accordingly, the present results would provide essential information to help in the conservation of the above species.

POSTER 44.

How urbanization affects multiple dimensions of biodiversity in tropical butterfly assemblages

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In the present study, we evaluated how taxonomic, phylogenetic and functional diversities of butterfly assemblages are affected by an urbanization gradient in Campinas municipality, Brazil. A dataset of Nymphalidae species representing different clades in 15 fragments distributed across urban, suburban, and rural sites was characterized and analyzed. For each site several environmental variables related to urbanization were considered, showing that disturbance level and patch connectivity mostly drove the environmental variation across the urbanization gradient. Taxonomic diversity increased towards more connected fragments, and phylogenetic and functional diversity did not vary in relation to urbanization. Nonetheless, some community-averaged traits responded to urbanization. Hind wing:forewing area ratio and frequency of lichen-like wings were positively related to urban fragments, while the frequency of brown wings, presence of ocelli and blue-green iridescence were related to suburban and rural fragments. The suitability of highly interconnected rural habitats for the maintenance of butterfly diversity is a result highly expected to be found. Nonetheless, our results showed that suburban sites are also important repositories of taxonomic diversity of butterflies, and maintain butterfly populations showing ecological traits related to forested habitats. A careful management of these fragments is important for the maintenance of high butterfly diversity levels, showing that increasing patch connectivity might be important to keep the metacommunity dynamics through the urbanization gradient. Suburban fragments may be important components of the urban matrix in terms of biodiversity conservation, forming a green belt across that fragmented landscape. The present results offer new opportunities to investigate functionality and life history traits of tropical insects and contribute to a better understanding of the ecological factors determining butterfly diversity patterns in highly disturbed tropical habitats.

POSTER 45.

Modeling the spatial distribution of *Symmachia arion*, an endangered butterfly from the Brazilian Atlantic Forest

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The Brazilian Atlantic Forest has high levels of species richness and rates of endemism, being considered a hotspot of diversity. Nowadays, less than 7.5% of their original extension remains into small and sparse fragments, highlighting the importance of its conservation. About 40% of Riodinidae butterflies already recorded for the Brazilian Atlantic Forest are endemic to this biome. *Symmachia arion* (Felder & Felder, 1865) (Lepidoptera: Riodinidae) is distributed in the southern portion of the Brazilian Atlantic Forest and is considered vulnerable in the red lists of threatened species of Paraná and Rio Grande do Sul states, south Brazil. The aims of this study were to estimate the spatial distribution and register new records of *S. arion* for the Atlantic Forest. We obtained data from literature and examination of specimens in museums to perform the modeling. A total of 15 variables (climatic and topographic) were selected (at a spatial resolution of 2.5 arc minutes), and Maxent algorithm was used for modeling the distribution of this species. Two new records were obtained for the extreme southern Brazil, which increased its geographic distribution in about 600 km. The area under the curve (AUC) was 0.93, indicating an excellent performance of the generated model. Furthermore, it was possible to locate 14 Federal Protected Areas that are included in localities of high probability of occurrence of *S. arion*. This method was effective to demonstrate the spatial distribution of this endangered species, aiming to increase the knowledge on its occurrence and also to direct sampling efforts in areas where remnant populations of *S. arion* probably occur.

POSTER 46.

Local habitat patch conditions correspond to patterns of neutral and adaptive genetic variation in naturally fragmented populations of a butterfly

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Preservation of large, well-connected areas of habitat can help to maintain high levels of intrapopulation genetic variability for species experiencing habitat fragmentation. However, for species that occupy naturally fragmented habitats, where populations have historically been somewhat isolated, genetic diversity may be more sensitive to local habitat patch characteristics (e.g., habitat patch size and quality) than to changes in the surrounding landscape (e.g. increased isolation and loss of potential habitat). We tested this hypothesis using local populations of the bog copper (*Lycaena epixanthe*), a butterfly endemic to naturally patchy, temperate Nearctic peatlands. Based on 190 repeatable and polymorphic amplified fragment length polymorphism (AFLP) loci, we found that populations of the bog copper exhibit high levels of genetic differentiation, and that genetic diversity within populations is better explained by local habitat patch descriptors (i.e., total patch size and proportion of open water) than by measures of landscape structure related to habitat isolation. Using an AFLP-based genome scan approach, we also identified signatures of selection in the bog copper that were associated primarily with local habitat conditions. Our results confirm the hypothesis that for species with a history of living under highly isolated conditions, the geographic distance separating habitat patches and the amount of nearby habitat has little influence on the genetics of local populations. For such naturally fragmented species, long-term persistence may therefore depend more on local habitat patch characteristics, such as patch size and resource availability, than on landscape connectivity.

POSTER 47.

Check list of butterflies of the Shahjalal University of Science and Technology, Sylhet, Bangladesh

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Butterflies (Lepidoptera) are the most studied order in the class insect. Also, butterflies are potent pollinators and good ecological indicators. In current research, a study on diversity of butterfly fauna was carried out in the Shahjalal University of Science and Technology (SUST) Campus of Sylhet, Bangladesh. A total of 38 species of butterflies belonging to the seven families were recorded in the study site during February 2014 to April 2014. Among the butterfly species maximum species were recorded of Family Nymphalidae (23.68%) followed by Lycaenidae (21.05%), Danaidae (13.16%) Pieridae (13.16%), Satyridae (10.53%), Papilionidae (10.53%) and Hesperiidae (7.90%). Out of 38 species Indian Cabbage White (*Pieris canidia*) was the most common species followed by Grey pansy (*Junonia atlites*), Common grass yellow (*Eurema hecabe*), lemon pansy (*Junonia lemonias*) and yellow pansy (*Junonia hierta*). This is the first approach to annotate the butterfly diversity in this area and future exploration will be continued to update the check list.

POSTER 48.

Land abandonment in Eastern Mediterranean – effects on butterfly communities

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As elsewhere in Europe, the Southern Balkans, part of the Mediterranean global biodiversity hot-spot, currently undergoes rapid land use changes, including abandonment of formerly pastoral lands, followed by successional encroachement. We investigated the effects of land abandonment on butterflies by examining the species richness and community composition in three countries differing in socioeconomic conditions (Greece: modernisation long underway, Bulgaria: current abandonment, Macedonia: traditional farming prevails). Total of 150 1 ha sites (5 regions 3 successional stages 10 replicates) were visited four times in 2012-13, covering main seasonal aspects; 129 butterfly species were recorded. The species richness was negatively affected by increasing cover of shrubs and trees, and the three successional stages differed in species composition. Several life history traits exhibited a significant relationship with the abandonment after taking into account geography and landscape covariates and phylogenetic relatedness among species. Abandoned sites were more likely inhabited by species overwintering in earlier stages, having fewer generations per year, non-migratory species and species with European range. More importantly, such sites were more likely inhabited by species with European or Eurosiberian (i.e., northern) distribution ranges, and less likely by Mediterranean endemics, but also by wide-ranging Holarctic species. It follows that open landsapes and sites are important for preserving species with restrited Mediterranean distributions and that conservation measures should support conitinuation of traditional land uses.

POSTER 49.

The Grayling butterfly along the Belgian coast: patch characteristics and landscape connectivity

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In NW-Europe, habitat loss and fragmentation are important causes of declines in biodiversity. Many species are nowadays unable to persist in increasingly smaller habitat patches with a declining habitat quality. The dunes along the Belgian coast are a suitable model system to investigate the effects of fragmentation, habitat loss and deteriorating habitat quality in a metapopulation context using the Grayling butterfly *Hipparchia semele* as a model organism. In 2003, 2004, 2012 and 2013, we mapped its presence in 115 potentially suitable dune patches along the western part of the Belgian coast. For all patches, we measured area, distance to the nearest patch, amount of Marram (*Ammophila arenaria* – the host plant of *Hipparchia semele*), trampling intensity (of both tourists and grazing animals), natural sand dynamics and the distance to the sea. The probability to find Grayling butterflies increased with patch size, the abundance of Marram, proximity to the sea and to the nearest suitable dune patch. Trampling intensity negatively affected the presence of the butterfly. Colonization events of the Grayling mainly occurred in large patches with a relatively high trampling intensity and close to the nearest dune patch and close to the sea. Extinction events, on the other hand, occurred in patches that were far from the nearest dune patch, at a larger distance from the sea and in patches with a high trampling intensity. We also calculated yearly connectivity measures for each patch taking distance, area and the mobility of the Grayling into account. Since 2003, some of the central patches in the Grayling metapopulation along the Belgian coast showed a strong decline in connectivity value. Conservation measures should, therefore, focus on restoring the habitat quality of these patches, on enlarging small habitat patches and on creating new suitable patches in the vicinity of these patches to facilitate colonization.

POSTER 50.

Diversity of swallowtail butterflies on non-Amazon South American ecoregions: elements of species composition, characterization of biogeographic units and evaluation of conservation status

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South American biomes have been considered a biodiversity hotspot for many butterfly groups, yet many of them have their biodiversity poorly known and are under conservation constrains due habitat conversion. Here we compiled information on the diversity of swallowtail butterflies (Papilionidae) on several non- Amazon ecoregions, measuring the resemblance among localities with vegetation types, climate and soil use, thus evaluating the environmental factors responsible assemblage structuring. Plus, data was analysed under the conservation perspective. Database was gathered from entomological collections and butterfly inventories, and mapped using GIS tools. Nonmetric Multidimensional Scaling was used to assess similarities among the best-sampled localities, and Principal Coordinates Analysis to obtain information on climatic effects. Conservation status was analysed comparing historical diversity hotspots with present day soil use and landscape heterogeneity. We obtained 4,033 records, including 111 species, representing 21% of Neotropical fauna. The number of species increased towards the Tropic of Capricorn, and richness was congruent with the latitude, ecoregions, vegetation type and soil use. The most diverse localities were native forest ecoregions from Atlantic Forest biome, and the less diverse managed grassland from Pampa. Localities were associated with the distance from the ocean, temperature range and precipitation; northern places were positively correlated with temperature, while southern localities were negatively correlated with temperature range. Regarding the conservation status, species richness was associated with landscape heterogeneity and connectivity, reflecting the role of natural disturbance on swallowtail diversity. The major effect influencing swallowtail diversity was habitat conversion into soybean, rice and eucalyptus monocultures. The data presented here suggest that conservation of South American swallowtails requires maintenance of resource heterogeneity and connectivity among habitat patches. Landscape features should provide background for developing suitable conditions and resources to enhance diversity in preserved areas surrounded by farming and livestock environments.

POSTER 51.

Mind the gap: towards the filling of butterfly biodiversity information using species distribution models and geographic information systems tools

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The most challenging issue on biodiversity analysis is the lack of information among localities, since samplings are concentrated to places where fauna have been inventoried. Here, we evaluated two methods to fulfil the lacking of information on butterfly diversity: species distribution models (SDM) and interpolation procedures from occurrence data. Species records were gathered from collections/inventories and mapped with GIS. To reduce the taxa number, only records of Papilionidae were selected. Analyses were conducted at two scales: biome (Pampa) and ecoregion (Uruguayan Savannah). SDM were performed extracting Worldclim environmental data from occurrence points. Environmental layers were selected by PCA, choosing layers not correlated that explained 90% of variation. Layers were clipped for Neotropical region and five algorithms applied using OpenModeller v.1.4.0: Bioclim, GARP, Maxent, SVM, Environmental Distance. SDMs were built for each species by selecting 70% of dataset as training and 30% as test samples. Resulting SDMs were adjusted to the minimum training presence and combined by ensemble forecasting; after they were clipped by Pampa and Uruguayan Savannah shapes. The interpolation method was performed mapping species occurrence in one-degree grid cells. Were selected 15 localities from Pampa and 8 from Uruguayan Savannah. To fulfil the gap between empty cells we applied thin-plate smoothing splines procedure using ANUSPLIN v.4.4. We gathered 2,036 records (36 taxa for Pampa, 25 for Uruguayan Savannah). SDM increased the list of taxa for both scales, while the interpolation did not altered richness. SDM also increased the estimates of richness per cell, while interpolation produced the opposite effect. SDM was more sensitive to ecoregion scale, while interpolation was more affected on biome. Both methods proved to be useful to fulfil gaps on butterfly diversity information. SDM are time consuming, but less sensitive to environmental heterogeneity; interpolation method is faster, but subjected to bias when landscape are not homogeneous.

POSTER 52.

Host plant preference in *Euphydryas aurinia* in Estonia

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Regionally polyphagous butterflies are often found to be host plant specialist at the local level, and regional information about species' ecology must be supplemented by local data to properly inform conservation actions. Host plant use of the endangered butterfly *Euphydryas aurinia* was investigated exploring both host preference of ovipositing females, and larval growth performance. Our results show that *Succisa pratensis* is the main host plant in Estonia. *Knautia arvensis* may serve as an alternative host plant but is clearly of inferior quality. In contrast, no species of *Plantago* were found suitable for larval development. *E. aurinia* appears thus to be a host specialist in Estonia even if the regional information available would have suggested a lesser degree of specialization. As a methodological contribution, our study stresses the importance of choosing appropriate methods for studies of oviposition preference. Multiple choice experiments in cages of 45x45x55 cm proved unsuccessful as butterflies failed to fly actively and did not make easily interpretable choices. In contrast, we demonstrate the suitability of an alternative method for observing female host preference (Singer, M.C. 1992 Anim. Behav. 44, 463– 471), previously applied to different species of *Euphydryas*.

POSTER 53.

Variable rates of response by Lepidoptera species to climate change

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Recent research on the ecological consequences of climate change has demonstrated that a wide range of species have, on average, shifted their distributions towards the poles and to higher elevations. However, individual species vary greatly in their rates of range expansion. We determined whether this variation could be explained by the sensitivities of species to different components of the climate. We produced population trends for 25 butterflies and 149 macro-moths over the past four decades in the UK, and related species' population trends to climate variables representing seasonal mean temperatures and rainfall. We used the goodness-of-fit of these relationships to assess species' sensitivity to climate, and then compared species' sensitivity to their observed distribution changes between 1970 and 2010. We demonstrate the effect of climate sensitivity of species on the magnitude of their distribution shifts, and discuss how our results can be used in understanding the fundamental limits on species' distributions and in devising evidence-based conservation strategies. Ongoing work is investigating the role of habitat and species' attributes in explaining additional variation in observed range shifts.

POSTER 54.

Determining the effects of monarch butterfly migration and range expansion on population structure

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Monarch butterflies (*Danaus plexippus*) are best known for their Fall migration from eastern North America to Mexico; however, they also occur as migrants in western North America and as non-migrant populations throughout the world. How do these different migratory strategies affect population structure and differentiation? To address this question, we used population genetic methods and microsatellite analyses. Rather than overwinter in Mexico like eastern monarchs, western monarchs overwinter at sites along the California Coast. Surprisingly, our analysis has shown that in spite of traveling to different overwintering sites and being separated by the Rocky Mountains, there is a lack of genome-wide genetic differentiation between these two migratory groups. In addition to residing in North America, monarch butterflies have dispersed throughout the world within the last 180 years, forming non-migratory populations. Despite this recent colonization, our research shows high levels of genetic structure and differentiation across areas and continents that are separated by bodies of water. Our work also suggests that genetic drift plays a major role in shaping allele frequencies in these newly colonized areas, through multiple and serial founder effects. In contrast, many of the monarch populations connected by land (including United

States, Mexico, Costa Rica and Belize) show limited levels of differentiation, likely because of the great ability of North American monarchs to migrate long distances across land. In addition to further demonstrating the impact of migration on gene flow and its prevention in population structure formation, this work is also relevant for conservation, as connectivity impacts population size and dynamics.

POSTER 55.

How pine silviculture and Atlantic Forest habitats shape butterfly diversity: a conservation approach

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This work aims to study the influence of *Pinus elliotti* forestry in the structure of butterfly assemblages in comparison with two natural habitats in the Atlantic Forest: Araucaria Forest and Altitudinal Grasslands. Data were collected between July/2007 and May/2008 in a protected area and its boundaries in extreme southern Brazil. Richness, abundance and species composition were evaluated, through individual-based rarefaction, ANOVA, PCoA based on Morisita similarity, ANOSIM and Renyi's entropy to assess the diversity for each environment. Data from inventories carried out in the 1950's in these same localities were also compared to evaluate the effects of silviculture in Satyrini species inhabiting altitudinal grasslands. Overall, 2647 butterflies distributed in 155 species were recorded, with *Pinus* sites differing from other sites in all diversity parameters, showing lower richness and abundance, and higher dominance and turnover in species composition. *Pinus* sites also presented more generalist and common butterfly species. *Araucaria* forest had higher richness, but did not differ from grasslands in abundance. There is segregation in composition among the three habitats, showing that each had a specific subset of species. Altitudinal Grasslands stand out, with several exclusive species. Among them, the species of *Pampasatyrus*, recorded 65 years ago have not been found, probably due to the replacement of grasslands by *Pinus* plantation in the last decades. The results suggest that *Pinus* forestry should be considered a less than ideal environment for butterfly fauna, favoring common eurytopic species. Moreover, the replacement of natural Grasslands by *Pinus* displaced several endemics such as *Pampasatyrus*, usually causing local extinction of these important taxon.

POSTER 56.

Evaluation of the fauna of frugivorous butterflies through three sampling procedures in southwestern Amazonia, Brazil

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Frugivorous butterflies can be easily sampled with attractive traps, thus facilitating standardized designs and allowing comparisons across space and time. However, in rich tropical forests sampling just one substrate - often the understory - can result in underestimation of the diverse fauna. We studied the fauna of frugivorous butterflies in areas of the Amazon rainforest, in Porto Velho, Brazil, focusing on the patterns of the taxa and the sampling procedures as well. From October 2010 to June 2012, four areas were sampled quarterly. In each area six transects were set. Three sampling procedures were used: traps in the canopy (C), traps in the understory (U) and entomological net (N). Sampling effort per transect was six traps for each C and U, and one hour for N. ANOVA was performed to assess the differences in richness and abundance among the sampling procedures. We recorded 1500 individuals (C = 403, U = 696, N = 401) and 142 species (C = 77, U = 94, N = 87). No difference was found in richness ($p = 0.148$) but abundance was significantly distinct ($p = 0.022$) among the sampling procedures. Cluster analysis (Bray-Curtis) evidenced samples obtained with nets were very distinct from those from the traps (20% similarity); between C and U the faunas are more similar (40%). Satyrini was the taxa with lower proportion of individuals in D (14,5%), while Coeni had the higher proportion (62%). Charaxinae was the least represented in N (13%). The data showed the importance of using different procedures for a better understanding of the fauna of heterogeneous environments such as the Amazon forest. Although attractive traps are efficient for sampling frugivorous butterflies, we reinforce the importance of sampling both strata and propose nets may add a great contribution to maximizing data in studies of diversity.

POSTER 57.

The "Butterfliess of Rio Grande do Sul" Programme – 21 years of research in South Brazil

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Rio Grande do Sul (RS) lies about 30 S latitude, at the extreme south of Brazil, in the transition of tropical and temperate climates, embracing the Atlantic Forest and Pampa Biomes. RS biodiversity has long been under severe threat from intense habitat conversion and fragmentation, agriculture, livestock and sylviculture. Nonetheless, these impacts on insect communities remained unknown, because the vast majority of the fauna has not been inventoried.

Up to the nineties, information about the RS Lepidoptera was fragmentary and mostly anecdotic, thus hindering comparisons over time and space and, thus, monitoring and conservation. In 1993, the research programme “Butterflies of Rio Grande do Sul” was devised to fill this gap, studying the biodiversity, communities and populations of butterflies by standardized methodology. It is aimed to survey species through the different ecoregions, prioritizing Conservation Units and previously unstudied areas. Lists of species are compiled and assemblages diversity analysed by habitat and environmental variables in search of occurrence and distribution patterns and processes. Modelling techniques are targeted to identify and predict potential areas of distribution and threats to the species in face of environmental change. After 21 years of studies, projects were developed in over 40 areas and more than 100 sampling sites. Lepidoptera Collections with over 14,000 specimens in two Universities were established. A Database, BorbRS2/ BorbBr, was developed. Over 800 species have been recorded and we estimated more than 1000 to occur in RS. Different faunas were evidenced for Atlantic Forest and Pampa. Particularly, the fauna from grasslands demands attention: very few areas are under protection in these habitats. From 2010, we joined efforts with fellow Brazilians also researching on butterfly diversity and conservation, creating the RedeLep. Finally, environmental education has also been catered for with field guides and subsidies for environmental management and conservation provided.

POSTER 58.

Vertical stratification of fruit-feeding butterflies community in the Brazilian Atlantic Forest

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The variation of abiotic factors in a vertical gradient can effectively generate different patterns of vegetal structure in tropical forests. The distribution of food resources in tropical forests may vary vertically according to the microclimates generated by forest structure along this gradient. The insects that consume these resources will consequently be organized in a stratified manner along this vertical gradient. Due to historical and geomorphological processes, several physiognomic aspects of the Brazilian Atlantic Forest are clearly different from those of other tropical forests such as secondary vegetation in distinct stages of succession and a lower canopy, resulting in a short distance between understory and canopy. This study aimed to describe the structure of a community of fruit-feeding butterflies in a region of Atlantic Forest in Southeastern Brazil, focusing on the foraging activity of this guild in two vertical strata. In one year of samplings, we observed that butterflies abundance and species richness were both higher in the canopy, and in the colder months this stratum maintained a regular number of individuals while abundance in the understory fell sharply. The species composition differed between canopy and understory, with 20 out of 69 species foraging predominantly at one stratum. Satyrinae were usually more common in the understory, whereas Charaxinae and Nymphalinae clearly prevail in the canopy. The tribe Satyrini appeared as strongly associated with the canopy, contrasting to the patterns obtained in other tropical forests. Sunlight incidence with consequent difference in temperature and biotic factors such as functional morphological traits may be selecting certain species or lineages to occur in a given stratum. Our study is the first to demonstrate a vertical stratification of fruit-feeding butterflies in the Atlantic Forest, a pattern already reported for tropical forests with higher canopies, highlighting the importance of sampling in this stratum.

POSTER 59.

Population genetics and the conservation of the endangered butterfly *Parides ascanius* (Lepidoptera: Papilionidae) in Brazil

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Parides ascanius was the first invertebrate officially recognized as being threatened in Brazil, in 1972. The species is endemic to the sand forests (known locally as “restingas”) of coastal Rio de Janeiro. Surrounded by the second biggest city in Brazil, it is found today in highly disturbed areas around the city and the Guanabara Bay. We developed a microsatellite library of 21 SSRs based on an enrichment-cloning protocol. Eight SSRs presented polymorphism, but one – which amplified two different regions on the genome – was discarded. We sampled one leg of males from eight populations (n=15-20), extracted total DNA and genotyped these samples using fluorescent primers and 3500x gene analyzer protocol. We analyzed data to produce FST metrics (using Fstat and hierfstat), a table of migrants (BayesAss), an assignment statistics (Adegenet’s DAPC), and to test for global versus local structure (Adegenet’s sPCA). FST and endogamy coefficient values are FST=0.0841, FIS=0.220 (hierfstat), =0.097, f=0.201, and mean allele richness across loci R=4.87 (Fstat). All eight populations are connected by migration, and seven populations have a relatively low chance of permanence (68-81%). In one population, isolated from all other populations by urban landscapes, this chance was as high as 88.9%. sPCA analysis shows a global structure around the city of Rio de Janeiro and Guanabara Bay, and the assignment statistics recovers eight clusters: seven well distributed among sampled sites and one, less widespread, corresponding to the isolated site. These findings are consistent with a natural scenario of metapopulation dynamics for P. ascanius, with low genetic diversity and high endogamy; but progressively isolated by the metropolitan matrix. Conservation efforts should: reconnect the isolated population, broaden searches for new populations, and preserve all extant habitat patches where *P. ascanius* still occurs.

POSTER 60.

Patterns of beta diversity of butterfly assemblies from the Southern Brazilian Atlantic Forest

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The Atlantic Forest domain is one of the 25 global biodiversity hotspots which supports high degrees of species richness and endemism. At its southern limit, heterogeneity in vegetation types is evident, from the coast into the continent: at east, Coastal Forest formations with Swamp Forest, followed by Subtropical Atlantic Forest, Araucaria Moist Forest and, further west, Seasonal Forests. Here we compared the patterns between butterflies communities among three phytogeographic provinces – Atlantic, Araucaria and Parana Forests - in Southern Brazilian Atlantic Forest and investigated how beta diversity may show diverse patterns for different taxonomic groups. Six data sets representing the above mentioned types of vegetations were obtained from the “Borboletas do Rio Grande do Sul” Research Program database. A matrix with a total of 628 species was compiled and, to assess whether phytogeographic provinces and/or type of forest would result in characteristic butterfly assemblies, similarity was evaluated for the assemblage as a whole and separately for each family. Non-metric multidimensional scaling ordination evidenced no grouping in relation to these variables; rather, it seems that distance between sites and sample size impacted more the results obtained when taking the whole data. When butterfly were analysed independently, the same pattern was observed for Nymphalidae, Lycaenidae and Riodinidae. Hesperiidae, Pieridae and Papilionidae, on the other hand, did not show any clear trends, suggesting that other factors – seasonality, preservation status of the areas etc - may play an important role. The understanding of beta diversity patterns of these communities may demand a deeper analysis among all taxonomic groups, especially those species which have stricter requirements from their habitats.

POSTER 61.

The distribution of frugivorous butterflies in two biomes of extreme south, Brazil

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The extreme south of Brazil lies in a transitional zone between tropical and temperate climates (latitude ˜ 30 S) and embraces two biomes –Atlantic Rain Forest, at its southernmost limit - and Pampa, which continues through Uruguay and northeastern Argentina. Both biomes are under serious anthropic impact, with very few intact areas; grasslands among the most threatened ecosystems of world. The aim of this study was to compile available data about fruit-feeding butterflies from south Brazil and evaluate patterns of occurrence and distribution in these Brazilian Biomes. Data were obtained from collections, literature and data-bases, totaling 2089 records in 127 species (S), of which 1022 registers (S=102) were from Pampa and 1067 (S= 119) from Atlantic Forest. Satyrini had the highest number of species and records among taxa, followed by Biblidinae and Brassolini. The endangered *Dynastor napoleon* and *Pampasatyrus reticulata* were only found in Atlantic Forest. *Haywardella edmondsii*, also threatened, was recorded only once in Pampa (another single register is known for Uruguay). In spite of the differences the biomes, species richness per subfamily/tribe was similar, and so was species composition: 73% of fauna was shared between them. Satyrini had the lower similarity values (0,61). However, the distribution of information was not even. Pampa registers are mostly concentrated by the border of Atlantic Forest and vast areas of Pampa have no data at all. Data from this “transition” zone may possibly not represent the Pampa fauna as whole. Records for the Atlantic Forest are much more evenly scattered, revealing a much better sampling of the fauna in this biome. Still major gaps in the knowledge on the diversity of neotropical grasslands remain. These data are crucial for the understanding of the biota and proper management and conservation programmes. Efforts for sampling these poorly known areas of the Pampa are urgent.

POSTER 62.

To graze or not to graze? Overgrazing appears to be higher threat than abandonment

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Grazing is one of the most suggested tool for managing seminatural grasslands. Although, grazing has been shown to benefit plant communities, the studies focusing on Lepidoptera give varying results. Thus, to use grazing as a conservational tool for butterflies one needs detailed information how grazing influences butterfly communities. Current study focuses on seminatural calcareous grasslands – alvars in Estonia. Since 1930. the area of these thin soiled calcareous grasslands have faced three fold decrease and less than 2000 hectares are managed today. These habitats are also one of the most butterfly rich ecosystem – more than 60% of butterflies known in the country are present in these habitats. Current project was initiated to measure the impact of grazing on butterfly communities in order to make justified management suggestions to preserve butterfly diversity. Standardized transect count were carried out to determine butterfly composition. Each site was visited six times over two years to determine butterfly composition. All grasslands were divided into three categories based on grazing intensity: abandoned, low grazing and heavily grazed sites. In general species abundance dropped abruptly with increased grazing, being three times lower at heavily grazed sites, compared to control sites and sites with low grazing intensities. Furthermore, species properties differed – species inhabiting heavily grazed sites had in average longer flight period and were more mobile compared to species inhabiting sites with low grazing intensities. Both of these characteristics indicate that the few individuals observed at heavily grazed sites were actually immigrants and did not form populations there. The overall results emphasises the negative effect of overgrazing as this appears to be highly detrimental to butterfly communities. Thus constant low or rotational grazing is suggested as conservation measure for this type of grasslands.

POSTER 63.

Ash deposit as a stronghold for the critically endangered Grayling (*Hipparchia semele*) in the Czech Republic

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The Grayling (*Hipparchia semele*), a European endemic, used to be one of the most common xerothermophilous butterflies in the Czech Republic. In past decades, it has been gradually declining to the last two recent regions in the country, the similar decline is known from many other European regions. In Central Europe, it occurs in edges of sparse pine groves and dry oak and hornbeam woods and forest steppes, it is relatively common also in many postindustrial areas. In 2009, we discovered a rich population of the Grayling inhabiting the Tušimice ash deposit, Kada district, western Czech Republic. In 2010, we studied the species demography and habitat preferences. Surprisingly, we discovered a population of about 2000 specimens, many times richer than all populations of natural habitats in the region. We realised that the deposit offer the source population for adjoining natural sites which are currently in suboptimal conditions because of their successional overgrowing. Contrastingly, the unintentionally and spontaneously established habitat mosaic within the deposit is ideal for all aspects of the Grayling life cycle: Females lay eggs on disturbed bare spots, where larvae feed on disturbed grasses. Males perch at solitary trees, shrubs and concrete structures. Both sexes feeds on ruderalised spots and hide in solitary trees and shrubs during bad weather. Graylings inhabiting the Kada district constitute one of the two last viable metapopulations in the Czech Republic. The Tušimice deposit, hosting the source population of the area, is therefore crucial for the butterfly conservation on a national scale. Originally, the deposit should be technically reclaimed creating an afforested artificial hill. Considering our results, the restoration plans were changed with focus on near-natural processes resulting in nearly-natural dry grasslands with solitary woods, although the restoration success has to be still monitored.

POSTER 64.

Conservation biogeography of Afromontane butterflies in the Gulf of Guinea Highlands

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The Gulf of Guinea Highlands, the only large mountains in West/Central Africa, represent a centre of endemism and tremendous conservation importance on a global scale not only for butterflies. On the other hand, many montane butterflies of the area remain enigmatic, although a better knowledge of them can be crucial for their effective conservation. Currently, practically all recent conservation activities focus on larger patches of continuous forests, which were recently shown as potentially insufficient for at least some of the endemic butterflies. During recent years, we repeatedly visited many localities in the whole montane area, as well as collected data from available publications and main collections, and thus gathered considerable knowledge on the local butterfly species and communities. During our visits, we also sampled tissues of several montane species for studies of their genetic structure and reconstruction of their historical biogeography. Here, we will present analyses of montane butterflies communities of montane butterflies. In our analyses, we combined several approaches including species richness of individual localities, species-specific distribution patterns, and patterns of a genetic heterogeneity of selected submontane butterflies based on mitochondrial genes and microsatellites. As one of the main results, localities with the highest conservation value were identified and their status, in relation to current land-use and potential socio-economic conflicts, could be considered.

POSTER 65.

What are the mechanisms governing species-distribution patterns in Hong Kong urban parks?

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Urbanization ranks among the primary reasons for continuing global biodiversity decline. Ecologists have put great effort into improving urban areas for habitat and wildlife, especially butterflies. While many studies have examined the important predictors for butterfly species richness in urban habitats, few have investigated how these factors determine how the species are distributed. In this study we sampled butterfly communities in 19 parks in Kowloon (the most urbanized area in Hong Kong). Using a metacommunity framework, we then analyzed the relationship between the butterfly communities and potential environmental factors such as park area, isolation of park, host plant and nectar plant species richness. In this metacommunity context we distinguished between communities controlled by biotic interactions and abiotic factors based on random and non-random community structures. We included the response of different species to environmental gradients such that the most important factors controlling the species-distribution patterns could be found. In total, we observed 68 and 27 species of host plant and nectar plant in selected parks of Kowloon. The host plant and nectar plant metacommunities showed a quasi-Clementsian and random structure, potentially indicating an anthropogenic influence through the planting of ornamentals. During preliminary surveys, we recorded 10 butterfly species by Pollard Walk and 20 species opportunistically. For both datasets, the butterfly metacommunity displayed a random structure possibly highlighting a lack of importance of abiotic factors for urban butterfly distributions though we cannot rule out insufficient survey effort at this time. Understanding the mechanisms governing species-distributions will help in maximizing butterfly species richness in urban habitats and ultimately provide insights into the species and habitat patterns which underlie the conservation potential of urban areas.

POSTER 66.

Forest openings provide alternative habitats for grassland butterflies

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The shift from traditional land use to intensive agriculture during last century has led to a drastic decrease in the area of semi-natural habitats in Europe. This loss of semi-natural habitats poses a major threat to many butterfly species, including common and widespread species. Managed forests, containing various open landscape elements like clear-cuts, forest rides, road verges and power line corridors, may potentially offer alternative habitats for butterflies. To assess the importance of these forest openings for butterflies, we examined butterfly fauna in forest clear-cuts in Estonia, giving special attention to the butterflies traditionally considered as grassland species and wider countryside species. Species composition and richness was compared with the regional butterfly fauna, taking also into account butterflies' food plant specialisation and mobility. Our results show that, from the butterflies' perspective, forest clear-cuts can to a remarkable degree compensate for the loss of semi-natural grasslands, providing alternative habitats for a very high share of regional butterfly fauna.

POSTER 67.

Microclimate variability significantly affects the composition, abundance and phenology of butterfly communities in a highly threatened Neotropical dry forest

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In temperate areas, microclimate is a key factor affecting the population dynamics of insects, but very few studies have examined the potential significance of microclimate in diverse tropical insect communities. We quantified the diversity and structure of butterfly communities in 2 different microhabitats, over seasons, and examined which abiotic (microclimate) and biotic (vegetation structure) components affected community composition. The study was performed from June 2009 to May 2010 at a dry forest in Ecuador, a globally threatened habitat with high levels of endemism. Two transects were established, one in each microhabitat, where baited traps were located in the understory and canopy to record butterfly species abundance. Humidity and temperature were recorded during the sampling period and vegetation variables were measured. A total of 3,731 individuals representing 93 species were collected. Higher species richness and abundances were found during the wet season. Linear regression models and CCA analyses showed microclimatic variability and vegetation structure (e.g., vegetation density) were significant predictors of the composition and abundance of butterfly communities. Major lineages of butterflies differed in their response to microclimate and microhabitat, explaining some of the spatial variation in community structure. In addition, some of the most abundant species changed their microhabitat preferences in the dry season. The tight relationship between butterfly communities, microclimate and microhabitat suggests that global climate change and habitat alteration are likely to act synergistically on tropical dry forest insect faunas, and these processes should thus be considered together in predicting future impacts on biodiversity.

POSTER SESSION: Sexual selection

POSTER 68.

Visual mate detection in a territorial butterfly – the effect of object brightness and background

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Many animals search for resources such as potential mates or prey using a sit- and-wait strategy. The success of such a strategy will depend on factors that affect a perched animal's ability to detect, intercept and successfully pursue a passing resource item. Intrinsic factors, i.e. visual system structure and function, have received a fair level of recent attention but less is known about effects on object detection from a perch of extrinsic factors such as size, coloration and speed of a passing object and the background against which the object is viewed. Here we examine in the field how extrinsic factors affect the detection of butterfly models by perched males of the butterfly *Asterocampa leilia*. Territorial males in this species employ a sit and wait strategy in the detection of potential mates and intruding conspecific males. By varying the coloration and brightness of models and documenting the background against which they are viewed, we test the hypotheses that the perched male's ability to detect a passing object is affected by the brightness of the model and the background against it is viewed. We found a strong effect of model brightness with dark models being most likely to elicit a response. We also found an effect of background type with models viewed against a bright blue sky eliciting the highest response. Further, we used in-field radiance measurements to model the luminance contrast between butterfly models and background. The luminance contrasts matches the behavioral data and we argue that the results is explained by variation in luminance contrast where a high contrast between the object and the background facilitates visual mate detection. This study expands our understanding of visual system performance in a simple task under field conditions.

POSTER 69.

Captivity experiments and in silico simulations indicates that large males of *Dryas iulia* (Nymphalidae, Heliconiinae) have better mating success than their smaller conspecifics

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Mating success in butterflies is influenced by many factors, generally involving female choice or male contests. Thus, mating success is subjected to evolution by intersexual or intrassexual selection. Here we investigated if the mating success of *Dryas iulia*males is related to their wing size. First, we investigated the age- specific sexual latency and the sexual activity of both sexes during the first 10 days of adult life under captivity. Second, we designed single-choice experiments (one small male, one female; one large male, one female) and one double-choice experiment (one small and one large male, one female), under controlled conditions (naïve butterflies; five-day males and two-day females; 27m^{3}test cages) to test if males have distinct mating success regarding wing size. Third, we tested the mating success of males with different wing sizes regarding the ability to find females in virtual open-space environments (1 ha), using in silico simulations based in flight quasi-steady models. The results indicate that sexes differ regarding their sexual latency and activity. Females become sexually active sooner than males, but this period lasts significantly less. Captivity experiments suggest that wing size does not influence mating success of males, at least in small-scale environments. Also, males with different sizes do not behave differently during courtship, and the receptivity of the females did not differ toward males with different wing sizes. However, the wing size of males influenced the mating success at large environments. We found a positive strong correlation with wing size and the time needed for males to find females in virtual open-spaced environments. Since natural populations of *Dryas iulia* show evidences of stabilizing selection on wing size, we postulate that males are subjected to intrasexual selection regarding wing size, but also to natural selection regarding the costs of bearing extreme phenotypes.

POSTER 70.

How to resist the sexual attractiveness of a co-mimic?

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When speciation occurs in sympatry due to natural selection and the accumulation of divergent ecological traits, maintaining isolation in the face of gene flow implies the evolution of strong reproductive barriers. *Heliconius* butterflies exhibit bright colourful wing patterns called "multiple-effect traits" because they are involved in mate recognition and in divergent selection for Müllerian mimicry. Strong sexual selection based on this trait has been shown in many species of Heliconius and is the strongest isolating process between several pairs of sister-species. However, what happens when sister-species of *Heliconius* are sympatric and mimic each other? We might predict that wing pattern similarity due to mimicry should interfere with assortative mating and the speciation process. Here, we address this question by examining sexual preferences between two closely-related species, *Heliconius timareta thelxinoe* and its co-mimic*H. melpomene amaryllis*. Those two species hybridize at a low frequency in natural conditions and hetero-specific matings remain occasional in captive populations. To understand the processes underlying assortative mating, we conducted mate-choice experiments and demonstrated asymmetry in partner choice. *H. timareta* males approach but do not court hetero-specific virgin females, implying that proximal cues such as female pheromones are involved in their choice. On the contrary, *H. melpomene* males court both species equally but rarely achieve mating with *timareta* females. This suggests that female choice would prevent hetero-specific mating in this direction. Further analyses revealed differences in chemical components between species. We also explored sexual selection against hybrids and backcrosses, which may contribute to reproductive isolation between species, and showed an asymmetry in their mating success. Such asymmetry would imply that one direction of gene flow is easier, a relevant parameter to understand adaptive introgression in this clade.

POSTER SESSION: General butterfly biology

POSTER 71.

Peculiarities of the hindwing androconia of *Eryphanis* butterflies (Lep.: Nymphalidae: Satyrinae: Brassolini)

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Eryphanis butterflies are very common in the Neotropics, and are reared and exported in large numbers for display in live butterfly exhibits. However, the biology of the adults is poorly understood. As all *Eryphanis* have a crepuscular lifestyle, studying their habits is all but easy. Accepting the challenge, to make a start, we paid special attention to their courtship behaviour. Chemical communication, which is likely to be important, seems to differ from non-Brassolini: matings result from furious flights in swarms occurring only at dusk and dawn. Male *Eryphanis* possess two putative sources of pheromones: glandular pads on the abdomen, plus very conspicuous, fully exposed (i.e. unprotected) scale patches on the hindwings. In other Brassolini mechanical contacts (and likely chemical interactions) between their alar and abdominal organs can occur, but in *Eryphanis* the hindwing patches seem to act independently, as they cannot touch the abdominal pads. While we cannot yet contribute to the understanding of pheromone communication in *Eryphanis*, we demonstrate several morphological and chemical peculiarities of their hindwing androconial patches which are unique and not found in other butterflies: large scales with outgrowing crystals, filled with sugars and spherical particles, and emitting volatiles (presumed courtship pheromones). Some unexpected findings might indicate a new direction for pheromone research.

POSTER 72.

The fate of seasonal plasticity under relaxed selection

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Many organisms display phenotypic plasticity as adaptation to seasonal environmental fluctuations. Often, such seasonal responses entails plasticity of a whole suite of morphological and life history traits that together contribute to the adaptive phenotypes in the alternative environments. While phenotypic plasticity in general is a well-studied phenomenon, little is known about the evolutionary fate of plastic responses if natural selection on plasticity is relaxed. Here we study whether the presumed ancestral seasonal plasticity of the rainforest butterfly *Bicyclus sanaos* (Fabricius, 1793) is still retained despite the fact that this species inhabits an environmentally stable habitat. Being exposed to an atypical range of temperatures in the laboratory revealed hidden reaction norms for several traits, including wing pattern. In contrast, reproductive body allocation has lost the plastic response. In the savannah butterfly *B. anynana* (Butler, 1879) these traits show strong developmental plasticity as an adaptation to the contrasting environments of its seasonal habitat and they are coordinated via a common developmental hormonal system. Our results for *B. sanaos* indicate that such integration of plastic traits—as a result of past selection on expressing a coordinated environmental response—can be broken when the optimal reaction norms for those traits diverge in a new environment.

POSTER 73.

The rise and demise of the Glanville Fritillary: is Britain's rarest butterfly about to go extinct?

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Clarifying the mechanisms which underpin species distribution and abundance remains a key question in ecology, and both climate and resources have been implicated in many studies as constraining factors at a range of temporal and spatial scales. The Glanville fritillary is the rarest butterfly in the U.K. It disappeared from the mainland during the mid-19th Century and is presently confined to the soft cliffs and chines along the south coast of the Isle of Wight, where sites contain large amounts of host plant *Plantago lanceolata* and provide warm microclimates essential for larval development. During spring, the larval stages are highly conspicuous within a silken 'web', thus facilitating an accurate population census. Larval web numbers have been monitored across key colonies since the 1980's and populations have fluctuated dramatically during this time. However, surveys conducted over the last few years have shown that it has declined severely in both distribution and abundance, and it is currently on the verge of extinction. Recent research has suggested an important interplay between vegetation structure and climate variability in determining population density. Therefore, the recent succession of extremely warm springs followed by cold ones is likely to be a key driver in declines, although the lack of genetic variation between populations may also be a factor. By using 18 years of field surveys combined with Meteorological weather data I explore the reasons behind the dramatic population fluctuations and more recent decline, from parasitoids to climate change, and suggest priorities for conservation.

POSTER 74.

DNA barcoding of Iberian butterflies enables a continental-scale assessment of potential cryptic diversity

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Several studies suggest that cryptic species may be surprisingly common in given taxonomic groups, but the actual fraction of biodiversity represented by cryptic species, as well as its distribution across the planet and taxonomic groups are subject to debate. However, the need of detecting these overlooked species is higher than ever, not only because of implications for various fields of research, but also because the planets biodiversity is decreasing at an alarming rate and conservation-related decisions should be taken based on accurate data. In this context, DNA barcoding has rapidly become a widely used method for exploring biodiversity. We use this technique combined with the generalized mixed Yule-coalescent (GMYC) model to explore potential cryptic diversity in one of the best studies invertebrate groups, the European butterflies. Based on 5782 COI sequences representing 299 species (ca. 60% of Europes fauna), we found that 83 of the 299 species were each split into two to four GMYC entities. Fourteen of these species involved cases that displayed at least 2.5% genetic divergence to the nearest conspecific entity. While some of these splits may indeed represent cryptic species, it is worth noting that all these genetically unique entities could be regarded as evolutionary significant units (ESU) that can be highly relevant for fields such as phylogeography and nature conservation. They provide research guidelines and can act as models to study evolutionary and speciation processes, irrespective of their specific status. Despite the advantages provided by DNA barcoding, we also show that limitations caused by a single-locus approach can result in overlooked patterns and thus recommend the use of additional data (e.g. morphology, nuclear markers) for improved resolution. Overall, DNA barcoding combined with GMYC represents a cost-effective and reasonably fast approach that is useful as a powerful first step for exploring cryptic biodiversity across wide areas.

POSTER 75.

Factors affecting the distribution of two species of African *Charaxes*: the role of hostplant distribution, environmental covariates and biogeography

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The genus Charaxes Ochsenheimer, 1816 is known for its high species richness and the diversity of hostplant associations. Here, I analyse the role of hostplant distribution, environmental variables and biogeographic regions in limiting the distribution of two african species of *Charaxes*: *Charaxes cithaeron* (C. & R. Felder, 1859) and *Ch. xiphares* (Stoll, 1781). I use species distribution records from available maps, museums and collections, and environmental variables from worldwide climatic datasets, and apply a logistic regression based on presence records and available habitat represented by localities with records for other species of *Charaxes* and fit models with several environmental variables (abiotic component, A), potential distribution of known hostplants (biotic component, B) and biogeographic region (geographic component, G). We then evaluate the models according to AIC and calculate AIC-weights to contrast the different hypothesis. For *Ch. cithaeron* the model with biogeographic region and environmental variables (A+G) was clearly better than alternative models, with AIC weights of 93.9%, but for *Ch. xiphares* the full model (A+B+G) with hostplant distribution as a covariate had lower AIC and AIC weights of 53.9%, but the simpler model without hostplant distribution (A+G) was still a good candidate with 46.1% of the weights. These results suggest that for some species the distribution is positively correlated with hostplant distribution, and adding this covariate might improve model performance. This might be the consequence of different environmental variables influencing the biology of the hostplants and butterflies. Several gaps in distribution data for butterfly and their hostplants are evident, but resulting predictions are informative and agree with patterns suggested in the literature. Extensive testing of distribution model requires more standardized sampling effort in regions with poor representation in collections.

POSTER 76.

The need to integrate experts, web-based tools and communities to create a truly global biological information system for butterflies

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Since 1998 several projects and partnerships, such as GloBIS/GART and Taxome, and individual efforts from several academic or enthusiastic lepidopterologists, have attempted to compile available biological information on all butterfly families in web-plattforms. They provide detailed taxonomic and basic biological information, but most of them depend on the contributions of few experts and are either static or sporadically updated. Broad-scope platforms like Wikipedia and Encyclopedia of Life (EOL) have also assembled valuable information on taxonomy and systematics, conservation, ecology, and other useful topics. They tend to be more dynamic due to the contributions of several projects and enthusiastic users, and provide technical support and several web services and tools for interaction and editing, but have drawn less attention from experts, thus great amounts of information remain unvetted or show geographical and taxonomic bias of the contributors. We searched for 17398 species names in selected specialized and broad scope resources, and found more than 100000 BHL pages, 35000 EOL text data objects, 32000 wikipedia articles in 9 languages, 2094000 GBIF records, and 22000 hostplant records in HOSTS database. Wikipedia and Savelas' page have almost complete coverage of species, while EOL and BHL included around half of the species, GBIF 39% and HOSTS just 19.9 %. All sources, except HOSTS, include all subfamilies and similar proportion of species per subfamily. Papilionidae and Pieridae have better coverage and content quantity in BHL, Wikipedia and GBIF, but do not differ substantially from other families in EOL. Few species accumulate large content, while half of the species is either not included or has less than 10 pages, data objects or records. Integration of several sources and a more profound collaboration between entomological and technical experts and common users is needed to improve current taxonomical and geographical coverage and the incorporation of additional detailed information.

POSTER 77.

The role of hostplants, vegetation and climate in limiting the distribution of South African butterflies

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Species distribution is limited by biotic and abiotic factors and biogeography. We show that species distribution models based solely on the correlation with environmental variables might be misleading and that hostplant associations can play a large role in explaining observed patterns. We use data from the South African Butterfly Conservation Assessment (SABCA) and other biodiversity databases, and applied an Environmental Niche Factor Analysis to measure the marginality and specialization of butterfly species distributions in South Africa, and use permutation tests to evaluate the hypothesis that observed values are due to limitations in (a) the distribution of their known hostplants, (b) association with vegetation units, or (c) biogeographical units, and to test the potential confounding effect of (d) autocorrelation in observations. Environmental variables were selected for each species according to their life cycle or flight season information. Hostplant are known for 443 South Africa butterfly species, but only in 91 cases we had enough distribution data for both butterfly and their principal hostplants. For 33% and 4% of the species, the observed pattern can be partially explained by vegetation units or biogeographic region respectively, while hostplant distribution seems to be an important limitation for at least 24% of the species. Although more than half of the species seem to be limited by climate variables alone (rejection of alternative hypothesis a, b and c), only 21% have a significant marginal distribution in the environmental space after accounting for spatial autocorrelation (rejection of d). Patterns are similar for endemic (n=33) and non-endemic (n=62), but hostplant distribution is proportionally more important for specialists (48%, n=23) than for generalists (17%, n=72). This evidence suggests that the environmental variables are often only indirectly correlated with butterfly species distribution and a better comprehension of their biology and ecology is needed in order to better interpret spatial patterns.

POSTER 78.

A comparative perspective on life span in geometrid moths

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In addition to the obvious applied significance of longevity research, studies on life span have a major role in evolutionary ecology: the longevity of an individual must be causally connected to a number of other species-specific life history traits. Although in-depth studies on life span exist for some butterflies, other Lepidoptera have largely been neglected. Even more importantly, the vast potential of comparative studies on species-specific adult longevity has remained unexploited. This is partly due to difficulties in obtaining comparable longevity data, but also the scarcity of phylogenetic information available has been an obstacle. For about hundred temperate geometrid moth species, we collected experimental data on individual life span, derived a phylogenetic tree based on largely original molecular data and applied phylogenetically explicit comparative analyses. We will report data on the strength of phylogenetic signal in adult life span, as well as phylogenetically corrected correlations of this variable with a number of other ecological and morphological traits like body size, adult feeding behaviour, egg production strategy, the degree of larval polyphagy and phenolology of adult flight.

POSTER 79.

World record holder in proboscis length of butterflies: the Neotropical skipper *Damas immaculata* (Hesperiidae)

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We discovered the new record holder for proboscis length in the Tropical Research Station La Gamba (Puntarenas, Costa Rica). During an extensive study the proboscis morphology, flower use and nectar intake rates were compared in 370 representatives belonging to 75 species of a local community of Hesperiidae. One individual of *Damas immaculata* had the longest proboscis ever recorded in butterflies measuring 52.7 mm. In addition, single individuals of *Perichares adela*, *Saliana severus*, *S. salius*, and *Damas clavus* (all members of the Hesperiinae) showed a proboscis length of more than 50 mm that is longer than previously reported for butterflies. The proboscis of these skippers was found to be about twice as long as the body length. All skippers which possess such an extremely long proboscis visited flowers of *Calathea lutea* and *C. crotalifera* (Maranthaceae) for nectar uptake. The observations of the flower visiting behaviour revealed that these butterflies did not remove pollen from *Calathea* flowers. It was concluded that these skippers were nectar thieves specialized to particularly deep and nectar-rich corollae. Compared to Hesperiidae possessing a short- or medium- sized proboscis, the skippers with an extremely long proboscis were bigger and had higher nectar intake rates in feeding experiments. It can be concluded from morphometrical studies in Riodinidae that big butterflies possess a large suction pump in the head that may overcome the biomechanical disadvantage of an extremely long proboscis.

POSTER 80.

Evolution of morphological diversity and sexual dimorphism in *Argynnis* butterflies

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Natural and sexual selection strongly influence the evolution of morphological diversity, including intraspecific traits such as sexual dimorphism. The predominant theory regarding the evolution of sexual dimorphism is that directional sexual selection on male traits is responsible for divergence of phenotypes between the sexes. However, the role of natural selection on divergent female phenotypes was recently highlighted in mimetic *Papilio* swallowtail butterflies. In *Papilio*, female-limited mimicry, rather than directional selection on male traits, has largely been responsible for the evolution of sexual dimorphism. Here, we test whether this pattern of female divergence under natural selection is more widespread, or whether the pattern observed in *Papilio* is an artifact of the special case of natural selection for female-limited mimicry. Specifically, we test whether morphological evolution in unrelated and equally brightly colored *Argynnis* butterflies shows female-biased accelerated rate of morphological (wing pattern) evolution similar to *Papilio*, and whether this drives the evolution of sexual dimorphism. For this purpose, we built a multi-locus (mitochondrial and nuclear genes, ˜3kb), species-level molecular phylogeny of *Argynnis* to reconstruct the evolutionary history of sexual dimorphism using likelihood approach. On the resulting well-supported molecular phylogeny, we mapped the evolution of wing patterns and found out that male wing patterns were phylogenetically conserved in this entire genus. However, there were three phylogenetically independent instances of the evolution of sexual dimorphism where females had diverged from the ancestral bright wing colors to become cryptically colored (unrelated to mimicry). In one instance (in two species), sexual dimorphism was induced by evolution of female-limited mimicry. This suggests that the evolution of sexual dimorphism due to natural selection on female traits is not necessarily linked with specific selection for female-limited mimicry in butterflies. This opens up the possibility that this phenomenon is more widespread than previously thought, and deserves extensive consideration.

POSTER 81.

Evolutionary assembly of butterfly mimicry rings in the Western Ghats, India

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Mimicry is an adaptation in many animals to gain protection from predators. There are two types of mimicry: 1) Müllerian mimicry, where two or more poisonous species, called models, share a warning signal, and 2) Batesian mimicry, where a non-poisonous species mimics poisonous species and thereby gains protection. Mimetic communities, called mimicry rings, are formed when multiple sympatric models and mimics gain protection from a specific aposematic pattern. The evolutionary assembly of mimicry rings and their dynamics are poorly understood. To study how mimicry rings are structured and maintained, we defined butterfly mimicry rings in the Western Ghats of southern India, generated a multi-gene, well-resolved and well-supported species-level phylogeny of mimicry rings, and mapped the evolution of mimicry types on this phylogeny. We found that Müllerian models within a mimicry ring usually acted as co-models mainly because of phylogenetic closeness, whereas mimics in the same mimicry ring typically arrived at the mimetic wing patterns due to a remarkable convergent evolution in wing patterns. Compared to the diversity of mimicry rings in the Oriental and Eastern Himalayan Regions, the Western Ghats consisted of fewer number of mimicry rings, and on average fewer models and mimics per mimicry ring. Moreover, within mimicry rings shared between the Western Ghats and Eastern Himalaya, the Western Ghats rings were depauperate in species richness, especially in the number of mimics. This may perhaps be due to recent origins of the Western Ghats butterfly mimicry rings and a short timespan for which the mimicry rings have evolved, reflecting the long isolation and trickling colozation of the Western Ghats that the local butterfly fauna generally shows. Overall, these results suggest a strong but usually under-appreciated influence of history and biogeography on the evolutionary assembly and diversity of mimetic butterfly communities.

POSTER 82.

Mimicry on the wing: Flight morphology and kinematics in a mimetic butterfly and its model

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Batesian mimicry is known to exert very strong natural selection on visual resemblance between aposematic toxic prey and the species that mimic them. How do sexual and species differences between models and mimics influence other dimensions of mimicry such as flight and behavior? To find out, we recorded flights of the toxic *Pachliopta aristolochiae* butterfly and its mimic *Papilio polytes*. *Papilio polytes* is a female-limited polymorphic mimic, i.e., males are monomorphic and non-mimetic whereas females are polymorphic, with a male-like (non-mimetic) form and one or more mimetic forms resembling locally available *Pachliopta* models. We found significant differences in body weight, investment in thorax versus abdomen, and several important flight kinematic parameters between males and females of each species. Thus, the sexes of both species had their own flight morphological differences that affected their flight kinematics, suggesting that selection pressures faced by the sexes may be different. When we compared models (*Pachliopta* males and females), mimic (*Papilio* mimetic female form) and non-mimics (*Papilio* male and non-mimetic female form), we found that the mimic had diverged from its ancestral kinematic background (the non-mimics of the same species) to resemble the models for the majority of flight kinematic parameters that we studied. These included important parameters such as wing loading, maximum turning rate and average radius of curvature of the flight path, which may influence the speed and escape flight of butterflies. However, the morphology of the mimic was indistinguishable from its ancestral morphological background. That is, morphology of the mimic was conserved but its flight kinematics had evolved substantially under directional selection to match that of its models. Thus, female-limited mimicry in *Papilio polytes* extends to flight behavioral dimensions despite the mimic's ancestral flight morphology.

POSTER 83.

Taking stock: advancing taxonomic, ecological and conservation research on Indian butterflies

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An advanced understanding of taxonomy and natural history is essential for studying biodiversity and its evolution, distribution and conservation. To bring the taxonomy and ecology of Indian butterflies up to date and then advance it much further, we have launched major initiatives that will: (1) help more completely inventory India's butterfly fauna with taxonomic and phylogenetic research, and (2) develop modern databases on butterfly populations and distributions that will assist in identifying priority areas and species for conservation. For this, we are extensively collecting butterfly specimens that will represent specific and subspecific variation throughout the Indian Region. These specimens are maintained in a climate-controlled collections facility, whose main goal is to host a worldclass, modern research collection that includes geo-referenced data and a frozen DNA library for all the specimens, which are catalogued in a centralized electronic database. We have a wet lab to sequence this material as and when needed for specific projects. This collection and the associated infrastructure is used for our taxonomic, phylogenetic, population genetic and other evolutionary and ecological research. To satisfy the second goal, we spearhead a citizen-science project that maintains a peer-reviewed website called Butterflies of India (http://ifoundbutterflies.org). This website compiles spot records of Indian butterflies that are databased and mapped on the fly. This website has already reported six butterfly species new to India, and dozens of species rediscoveries and major range extensions, including that of many species that are legally protected. We hope that by intensively studying the Indian butterfly fauna in our lab and extensively engaging with the citizen science model, we will boost museum- and field-based scientific research on Indian butterflies. In addition, we closely engage with policy makers to convert our understanding of butterflies into tangible conservation action for the long-term preservation of insect diversity in India.

POSTER 84.

The role of volunteers in museum collections - a case study at the NHM, London

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Data collection and other aspects of long term projects is often costly and labour intensive. Therefore, in the current economic climate, volunteers are increasingly relied upon as an integral part of science projects across the board. The responsibilities may vary from low level tasks and general tasks to those such as data inputting and analysis which could have a great impact upon the accuracy of the results and output of the project as a whole. Today's volunteers encompass a range of backgrounds and motives; from the unskilled and amateurs to the ever increasing proportion that are highly qualified and with a strong ambition to continue a career in that field. The role of volunteers in field-based, data collection on conservation projects and their performance in subsequent data collection has been investigated, with some studies viewing the impact of volunteers in a positive light (1). However, this topic has yet to be explored within the meticulous and specialised area of museum curation. The Natural History Museum (hereafter referred to as NHM) in London, UK, contains a vast and historically important collection of over 700 million specimens and subsequently employs over 400 volunteers to aid long term curatorial projects and general museum function such as public engagement (2). The NHM Lepidoptera collections house c. 9 million specimens and utilise volunteers to aid long term projects including re-curation and databasing of specimens (3). Our main aim is to present a brief review of the impact of volunteers within NHM butterfly museum collections as a case study. Milestones and future directions will be also discussed. References: 1. Bildstein, K.L. "Long-Term Counts of Migrating Raptors: A Role for Volunteers in Wildlife Research." The Journal of Wildlife Management. Vol. 62, No. 2 (Apr., 1998), pp. 435-445. 2. NHM 2014a http://www.nhm.ac.uk/support-us/volunteer/. 3. NHM 2014b http://bit.ly/1k8VMTR

POSTER 85.

The importance of host plants of four endangered butterfly species

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In butterflies, host plant preference has frequently been shown to vary even at a limited geographic scale. This implies that conservation actions should preferably be based on locally derived information on a species' ecology. In order to evaluate the importance of host plant species as a determinant habitat quality, host plant preference was studied in Estonian populations of four butterfly species: *Lopinga achine*, *Lycaena dispar*, *Euphydryas maturna* and *Coenonympha hero*. We studied both oviposition preference of females, as well as growth performance of larvae on different plants. Females of *L. achine* did not discriminate between offered host plants. Although larvae preferred *Carex sylvatica* and *C. montana* over various grasses in choice tests, growth rate was almost equal on several host plants; with *Festuca ovina* being a clear exception. Consistently, survival rate was also lower growing on *F. ovina*. Multiple-choice oviposition experiments with *L. dispar* revealed no preference between *Rumex* species offered. The larvae showed a weak preference towards mostly *R. crispus*. In growth performance experiments all offered plant species were similarly suitable for the larvae of *L. dispar*. Survival of the larvae of *E. maturna* was similar on *Viburnum opulus* and *Fraxinus excelsior*, still much lower on *Melampyrum pratense*. However, growth rate did not differ between these plants. Oviposition experiments with *C. hero* suggested that physical chacteristics of oviposition substrate rather than host plant species are decisive in oviposition site selection. We found that various grass species of *C. hero* were similarly suitable in terms of larval growth performance. Despite of some differences in suitability of host plants studied, we conclude that all the four butterfly species are polyphagous enough, and their acceptable host plants widespread enough, so that availability of any particular host plant species is not likely an important component of habitat suitability.

POSTER 86.

The butterflies of Waterloo Region: Species scarcity rankings and a transect-based analysis along an urban gradient

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This study examines butterflies as indicators using a gradient-based study approach in the Region of Waterloo, Ontario, Canada. The urban gradient was characterized by Environmentally Sensitive Policy Areas (ESPAs), urban parks, golf courses, residential and industrial areas. An extensive review of existing data on butterfly presence/absence in the region was conducted to determine what species are relatively uncommon or rare in the Region and examine how butterfly presence/absence has changed over the last 80 years. Changes in butterfly abundance and diversity along a gradient of urbanization was also examined. Walking transects were established at fifteen sites, each of which represented a land use within the urban gradient identified. Shannon diversity scores were compared using a Kruskal Wallis test and indicated that species richness and evenness was significantly different between ESPAs and urban parks and compared to the remaining land uses. Overall butterfly abundance was observed to be highest in industrial areas but heavily influenced by counts of two non-native species. ESPAs were identified as 1) supporting the most diverse butterfly community out of the 5 land uses examined and 2) providing habitat for the highest number of rare and uncommon species, indicating that current regional policies in place for protecting rare species are effective. Through an extensive literature review it was concluded that butterflies are effective indicators in temperate regions within a small geographic area such region studied. Therefore it is expected that the results of this study indicate how other terrestrial taxonomic groups, which are known to show a similar response to urbanized land uses, may be impacted by urbanization in the Region. It is anticipated that the results of this study will guide regional land use planning as it identifies rare and uncommon butterfly species as well as what areas need habitat enhancement to support more diverse communities.

POSTER 87.

Cool caterpillars: measuring the cold hardiness of immature monarchs

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Eastern North American monarchs (*Danaus plexippus*) are well known for their long-distance annual migration from the eastern US and southern Canada to high elevation overwintering sites in Mexico, a journey that can cover over 4500 km. However, climate change models indicate these overwintering sites may not be suitable for monarchs within as little as 40 years. Citizen science reports have shown that monarchs are now present and breeding in the states along the US Gulf Coast throughout the winter, where these monarchs are primarily using non-native tropical milkweed (*Asclepias curassavica*) as their host plant. These Gulf Coast areas experience freezing temperatures and thus immature monarchs may be exposed to lethally cold temperatures. To test immature monarch cold hardiness, I exposed monarchs to freezing temperatures and recorded both the supercooling point (SCP) and lower lethal temperature (LT50) for different developmental stages. Median SCPs for immature monarchs range from -26.1 to -9.6 C, with eggs having the coldest SCP and third instar larvae having the warmest SCP. Larvae appear to be freeze intolerant, with the LT50s at temperatures near each stage's respective median SCP. However, eggs seem to be chill-intolerant, with an LT50 occurring at a temperature much higher than their median SCP. While most monarchs tested were summer monarchs lab-reared in southern US winter conditions, these results were further verified by both testing wild winter monarchs raised on tropical milkweed in Texas, as well as recording temperatures on monarch occupied milkweed plants at different Texas field sites. These findings can help inform future modeling and conservation efforts for monarchs throughout their life cycle, in particular guiding host plant restoration and habitat management in the southern US.

POSTER 88.

Diapause termination and post-diapause development in relation to phenological specialization in two temperate butterflies

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Spring phenology of winter diapausing insects is a net result of both diapause dynamics and reaction norms of post-diapause rate of development. Spatial variation in seasonality patterns results in an even more complicated picture of latitudinal gradients in insects' life cycles through evolution of local adaptations. We explored local adaptation in diapause duration and post-diapause pupal development in two butterfly species – univoltine *Anthocharis cardamines*, a phenological specialist, and bivoltine *Pieris napi*, a phenological generalist, that share their host species range but differ in life history biology. We found that diapause duration was shorter in *Anthocharis cardamines* than in *Pieris napi* throughout the explored latitudinal range. Moreover, in *Pieris napi* the diapause duration showed an increase from southern to more northern populations. The two species also responded differently to winter thermal conditions: additional lowering of temperature during the chilling period shortened diapause duration for *P. napi* pupae but not for *A. cardamines* pupae. Post-diapause pupal development was faster after longer periods of cold conditions and also faster in more southern populations of the both species. A cogradient latitudinal pattern in pupal post-winter development was shown in both species; however, they were due to different reasons. The cogradient pattern of *A. cardamines* was likely due to selection for phenological matching to its local host plant community. The latitudinal pattern in *P. napi* was primarily an effect of slow development of only a fraction of the pupae from the most northern population, which may be due to the presence of both bivoltine and univoltine strategies in the northern location. These differences in the emergence times were determined by the extended duration of quiescence period in *P. napi* and unequal rate of morphogenesis in *A. cardamines*. Thus, the two butterfly species had similar geographical patterns of pupal development but due to different reasons.

POSTER 89.

Lep-MAP: fast and accurate linkage map construction tool for large SNP datasets

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High-throughput sequencing technologies allow cost-efficient genotyping of millions of single nucleotide polymorphisms (SNPs) for hundreds of samples. However, the tools that are currently available for constructing linkage maps are not well suited for such large datasets. Linkage maps of large datasets are helpful in de novo genome assembly by facilitating comprehensive genome validation and refinement, as well as in family-based linkage and association studies, quantitative trait locus (QTL) mapping, analysis of genome synteny and other complex genomic data analyses. I will describe a recently published tool, called Lep-MAP (Lepidoptera-MAP), for constructing accurate linkage maps with ultradense genome-wide SNP data. Lep- MAP utilizes data simultaneously on multiple outbred families and can increase linkage map accuracy by taking into account achiasmatic meiosis, a special feature of Lepidoptera and some other taxa with no recombination in one sex (no recombination in females in Lepidoptera). Lep-MAP is also fast and memory efficient and largely automated, requiring minimal user interaction. Results with real and simulated data are presented, including a genome-wide linkage map of the Glanville fritillary butterfly (*Melitaea cinxia*) with over 40 000 SNPs. Also a sneak preview of new features in Lep-MAP2 are described is some detail. Lep-MAP is publicly available with its source code from http://sourceforge.net/projects/lep-map/.

POSTER 90.

Effect of annual weather variation vs. short-term weather extremes on population change of the alpine butterfly *Parnassius smintheus* in the Rocky Mountains

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We use estimates of annual population change (Rt) from 21 sub-populations of the alpine butterfly, *Parnassius smintheus*, over a 21-year interval to determine the relative importance of annual variation in weather for each month and short-term extremes in weather within months in determining population change. Variables include monthly estimates of mean, maximum and minimum temperature as well as extreme maxima and minima in each month. Snow variables include monthly snow amounts and date of spring snow melt. A combination of mixed effects models and regression trees were used to identify patterns of combination of significant interacting variables. Although population size dominated the effects on Rt in most models, there were key weather variables related to population change. In particular, especially cold Novembers, and extreme cold events within November, were associated with population decline, as was early snow melt in spring. The strong effect of population size on Rt (density-dependent population growth) was only apparent in years with relatively benign November weather. Results support the general pattern reported earlier of population declines in both especially cold winters and especially warm winters, likely through differing respective mechanisms.

POSTER 91.

Evolution and development of insect wings: A comparative analysis of the genome-wide targets of the Hox protein Ultrabithorax in *Bombyx mori*, *Apis mellifera* and *Drosophila melanogaster*

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Amongst various different insect groups, there is remarkable diversity in number and size of wings. The insect body is divided into segments where the fate of different organs is chiefly controlled by a set of highly conserved Hox master control genes which fine-tune certain pathways downstream to bring about the development or modification of different organs. Suppression of wing fate and specification of haltere fate by the Hox gene Ultrabithorax (Ubx) is a classic example of Hox regulation of serial homology. This differential development of wing and haltere in Drosophila represents the evolutionary trend that has established the differences between fore and hind wings in insects. Interestingly, Ubx protein itself has not evolved amongst the diverse insects. Ubx is expressed in the developing hindwings of the butterfly and *Bombyx*. Thus, the difference between lepidoptera hind wing and a diptera haltere may be due to a changing assortment of target genes that carry out different developmental functions. We are interested to see what developmental differences occur in the hind wings to that of the fore wings in lepidoptera in comparison with differential wing and haltere development in diptera. We have identified targets of Ubx in the hindwing of *Bombyx* (lepidoptera) and compared the same with those in *Drosophila* and *Apis* (hymenoptera). We have observed that Ubx targets in all the three insects share similar biological functions in comparable proportions. Wing related genes are enriched among Ubx targets, and a comparison suggests that Bombyx is more diverged from *Apis* than *Drosophila*. These targets, however, do not show differential expression in the fore and the hind wing in *Bombyx* as opposed to wing and haltere in *Drosophila*. We are exploring the possibility that diversity in morphology in insect wings may be at the level of evolutionary changes in regulatory sequences of these targets.

POSTER 92.

Metamorphosis revealed: micro-CT scanning as a tool for studying Lepidoptera development

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Studies into the morphological and anatomical nature of insect metamorphosis have traditionally relied almost entirely on dissections and histological sections of a few model organisms such as *Calliphora* (blowfies), *Drosophila* (fruitflies), and for Lepidoptera, *Manduca sexta* (tobacco hormworm). The advantages of such studies are that they are highly detailed and generally yield a wealth of information. The main disadvantages are very time consuming, they do not readily allow for broad scale comparisons across taxa, and it is difficult to extract quantitative data from the results. This makes it difficult not only to make comparisons between groups, but also to pinpoint the exact time for important developmental changes. Here we explore how micro-CT scanning can be used to examine *Vanessa cardui* (painted lady butterfly) throughout metamorphosis. We demonstrate how the results can be used to document and pinpoint key changes, as well as provide new insights into Lepidoptera metamorphosis and allow for quantitative measurements of trachea and gut volumes.

POSTER 93.

Virtual dissections: exploring micro-CT scanning as a method for non-destructive ‘dissection’ of valuable Lepidoptera material.

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Morphological studies and documentation of characters are of singular importance in Lepidoptera taxonomy and systematics despite the increasing popularity and importance over the past decade of molecular methods such as DNA barcoding. In particular, genitalia dissections of type material are often crucial in revisionary taxonomic studies. However, type material is not always readily available as the institutions holding the material may be reluctant to send it on loan, and even more reluctant to allow such material to be dissected. A possible solution to this problem could be virtual dissections through micro-CT scanning. Since it was first applied to the study of insect morphology more than a decade ago, micro-CT has been a state-of-the-art technique, particular in comparative studies of internal, soft-part morphology such as musculature. Here we demonstrate how a standard micro-CT scanner can be used to carry out highly detailed, non-destructive virtual dissections of Lepidoptera male genitalia. We demonstrate how freely available software can be used to examine the data and illustrate characters, and we propose a workflow by which data can be shared for analysis by workers across the world.

POSTER 94.

Habitat requirements of the endangered parasitic butterfly *Phengaris arion* close to its northern distribution limit

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Populations close to species distribution limits often differ in their habitat requirements from more central populations of the species distribution. Knowledge of species ecology derived from the latter may therefore not be sufficient to ensure successful conservation of peripheral populations. In this study, we examine habitat requirements of *Phengaris* (=*Maculinea*) *arion* (Lepidoptera, Lycaenidae), an endangered myrmecophilous butterfly, in populations close to its northern distribution limit (in Estonia). A particular emphasis is given to its interactions with other species – host plant and host ant use, and, as a novel aspect, adult predation by dragonflies. *P. arion* was found to be restricted to grassland patches with much higher host plant abundance than shown in other regions and predicted by habitat suitability models. All direct observations of the host ant use of *P. arion* were limited to the colonies of *Myrmica lonae*, an ant species which has nowhere else been demonstrated to be the primary host of this butterfly. Our data thus contribute to the emerging understanding that host ant use in *P. arion* can be geographically remarkably diverse. The results indicate that dragonfly predation on adult butterflies could be an essential driver of patch occupancy in *P. arion*. Our findings suggest that top-down influences, largely neglected in butterfly conservation, may actually need to be considered.

POSTER 95.

Sex chromosome dosage compensation in *Heliconius* butterflies

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Dosage compensation is the equalization of gene expression levels in response to differences in gene dose or copy number. Dosage compensation is classically considered to play a critical role in the evolution of heteromorphic sex chromosomes. As the X and Y diverge through degradation and gene loss on the Y (or the W in female-heterogametic ZW taxa), it is expected that dosage compensation will evolve to correct for sex-specific differences in gene dose. Although this is typically observed in male-heterogametic (XY) species, recent genome-wide expression studies in other taxa have revealed striking exceptions, especially in ZW taxa such as birds and snakes. In these taxa, the single Z of females is under-expressed relative to males. These results fuel speculation that incomplete dosage compensation may be a defining characteristic of female-heterogamety. However, Lepidoptera (moths and butterflies) are also female-heterogametic, and evidence is accumulating that at least some species show balanced expression between sexes on the Z chromosome, contradicting the emerging consensus that ZW taxa lack complete dosage compensation. We report the first genomic assessment of sex chromosome dosage compensation in butterflies, focusing on the genus *Heliconius*. Our results show that Z chromosome global expression is significantly lower than autosomes in both sexes, similar to previous reports in Silkmoth, and suggesting a novel mechanism of dosage compensation may exist in the Lepidoptera. We additionally detect a significantly greater global Z expression in males over females, indicating dosage compensation is imperfect in this species. However, the magnitude of this dosage effect is much less than the magnitude of reduced Z expression in both sexes. Methodologically, we introduce mixed-model linear regression with a poisson error structure as a useful statistical framework for assessing sex-chromosome dosage compensation.

POSTER 96.

Temperature and fire increase ant-tending of Fender's blue butterfly larvae

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Mutualistic interactions are conditional, their outcome dependent on the ecological conditions under which species live. Fire is a strong driver of community dynamics and the time since burning has a strong effect on abiotic factors and biotic interactions. While direct effects of fire on butterfly populations have been well documented, the degree to which fire changes species interactions has not been heavily emphasized. We examined one mutualism, that between larvae of the endangered lycaenid Fender's blue (*Plebejus icarioides fenderi*) and a variety of tending ant species. We quantified how this mutualism was affected by prescribed fire, designed to maintain Fender's upland prairie habitat. We found that fire enhanced larval survival two seasons after the burn. A study of Fender's host plants, Kinkaid's lupine (*Lupinus sulphureus* ssp. *kincaidii*) and spurred lupine (*Lupinus arbustus*) determined that elevated larval survival could not be explained by changes in gross plant chemistry, as there were no effects of fire on foliar C, N, P or water content. Fire also didn't affect the quantity of food. While there were more lupine leaves in previously burned plots, there was also enhanced oviposition, resulting in a nearly identical number of eggs per leaf. We identified ant tending as a potential driver for enhanced survival. Changes in soil temperature influenced ant recruitment time and burning affected the length of tending bouts. Fire has the potential to affect ant tending via changes in microclimate and through effects on larval attractiveness.

POSTER 97.

The Butterfly Guide: Developing mobile tools for biodiversity informatics and natural history education

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Biodiversity informatics is a growing discipline devoted to the collection and management of the world's biodiversity data. One area of active growth in this field is the development of mobile technologies aimed at natural history education, data collection, and citizen science. These tools could be useful for a variety of educators, land managers, and researchers, but often require prohibitive amounts of time and money to create. The Butterfly Guide: Butterflies of the Sacramento Valley, Delta, and San Francisco Bay Area is a new mobile biodiversity informatics app with which users can collect and share butterfly observation data. The app was conceived as a prototype and template for those interested in creating similar tools for biodiversity education and data collection, and all source code and development materials are freely available to other developers interested in creating similar field guides to different regions and/or taxa. By promoting the development and dissemination of free natural history education tools, this project aims to increase opportunities for non-scientists to participate in the scientific process, generate reliable biodiversity data, and engage in the natural world.

POSTER 98.

Climatic niche characteristics of the butterflies in Europe (CLIMBER) – a new database for measuring the response of butterflies to climatic changes

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CLIMBER is a newly published database providing information on the position and breadth of major climatic niche characteristics for almost all European butterfly species. It includes the mean annual temperature which forms (as 'species temperature index' = STI) the basis to calculate the 'community temperature index' (CTI). This index already proved to be a powerful indicator in the context of climate warming. In addition to temperature-based climatic indices, the database also includes several indices for water availability. Here, we present new applications of this dataset, which help to assess how butterfly communities react to climatic change. The results demonstrate that butterfly communities across Europe have already been responding to climate warming by shifting their composition towards warm-adapted species. Butterfly species assemblages are fast indicators of climate change and respond to temperature fluctuations with a time lag of only 1-2 generations, supporting the assumption that CTI increases are not merely caused by land-use changes. However, responses are smaller than anticipated from climate warming and differ among regions and according to species traits. Communities at lower latitudes and higher altitudes are changing less, and the same applies to warm-adapted specialists and forest species. A phylogenetic analysis of the CLIMBER dataset revealed that adaptations to cold thermal limits are phylogenetically conserved while adaptations to the warm limits are not. We assume that the phylogenetic signal for cold-adaptation is a legacy of the ice ages. The lack of evidence for phylogenetic conservatism of warm-adaptations may indicate different evolutionary or ecological processes at cold and warm range margins probably caused by increased importance of biotic interactions under warmer conditions. A potential quicker adaptation to warmer conditions or a lack of range-filling

at the warm ends due to biotic interactions together with anthropogenic landscape barriers to dispersal might lead to the observed climatic debts of butterflies.

POSTER SESSION: Butterflies as hosts

POSTER 99.

Beauty and the beast: Lepidoptera and their endosymbionts

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Advancements in molecular biology over the last 30 years enabled the revelation that symbionts are both extremely prevalent among arthropods and highly diverse. More recently, symbionts have been demonstrated to play an important role in host evolution and biology including reproduction, metabolism and behaviour. Despite reporting almost 200 species (>140 butterflies and >50 moths) known to carry one or more endosymbiont(s), most often *Wolbachia*, the literature is lacking more in-depth research on these symbiosis in Lepidoptera. This is particularly striking given the vast amount of interest and accumulation of data in this taxon on other aspects of their biology. As many Lepidopteran species are of ecological or economic importance, and with even more being endangered, it is critical that such influential elements be surveyed and investigated. With this poster we hope to raise awareness and feed discussion on such topic.

POSTER 100.

Encapsulation response and other immunological assays in the Glanville fritillary butterfly (*Melitaea cinxia*)

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Immunity is one of the most essential traits in all living organisms. Therefore, understanding how ecological, biotic and abiotic, factors that vary between populations impact individual immune system functioning in the wild is of crucial importance My PhD project aims to investigate ecological immunology in the Glanville fritillary butterfly (*Melitaea cinxia*). The main objectives of my thesis are: (i) to test how the different immune defense assays relate to each other; (ii) to assess within and among population level variation in individual immune defense within the metapopulation of *M. cinxia* in the Åland Islands; (iii) to investigate how individual immune response varies in relation to different ecological conditions (e.g. density, parasitism, inbreeding) that differ among local populations, and (iv) to understand how immune response covaries with other crucial life history traits, such as reproductive success and dispersal ability. In this poster, I will present some preliminary results of my first experiment. A functioning immune system is an extremely complex trait, and therefore costly to be maintained. Individual variation could be related to different levels of investment in immunity, and possibly to different strategies in immune response. I investigated the relationship among the different components of the immune system in the Glanville fritillary butterfly, by performing four immune assays commonly used with insects. These immune assays included total hemocyte count from larval hemolymph, phenol oxidase activity and lytic activity (both with larval and pupal hemolymph), and pupal encapsulation rate. Immune assays were compared between naïve individuals and individuals whose immune response was induced by non-pathogenic bacterial infection (*E.coli*).

POSTER 101.

Is the presence of *Wolbachia* responsible for the high species diversity within *Actinote pellenea* species complex?

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The presence of the endosymbiont bacteria *Wolbachia* is believed to induce speciation in insects through several mechanisms that generate reproductive isolation. These bacteria were already described in the nymphalid tribe Acraeini, however the results of symbiont-induced speciation on this butterfly species diversity were never investigated. *Actinote pellenea* Hübner [1921] (Nymphalidae: Heliconiinae: Acraeini) is the most diverse species within the genus, with 15 formally recognized subspecies, and, at least, five other awaiting for formal description. We sampled 53 individuals of *A. pellenea* from 20 localities in South America, especially in Brazil, to test taxa limits and the influence of the endosymbiont *Wolbachia* in shaping the genetic variability of this species. Two genes, the mtDNA gene COI and the nDNA gene EF-1, were amplified and sequenced to infer phylogenetic relationships among *A. pellenea* taxa. Relationships were estimated using by Maximum Likelihood analyzes, and *A. carycina* was used as outgroup. The genes wsp and gatB were used to infer the presence of and the relationship among *Wolbachia* found in each butterfly specimen. As expected, topologies for butterflies diverged when each gene was analyzed individually, and COI exhibited more gene diversity than the nuclear gene. Nevertheless, both COI and EF-1a did not recover subspecies as monophyletic groups. All, but three localities, were positive for *Wolbachia*, and specimens negative for these bacteria grouped together with individuals positive for their presence. These preliminary results raised uncertainties about the monophyly of the species, and the presence of *Wolbachia* may be responsible for the misperception regarding the relationships among its subspecies.

POSTER 102.

Immunological maternal effects in the Glanville fritillary butterfly (*Melitaea cinxia*)

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The evolution of the immune system is a crucial factor in the life of any species as parasitic infections may have a strong adverse effect on the host's fitness. Therefore there is strong selection pressure on a highly effective immune system that is able to discriminate between self and non-self. Environmental conditions are also known to have a great impact on individual's immune defence. However, it is not only the environmental conditions the individual experiences at present that are important, but also those that it experienced during its development or even those experienced by its parents (i.e. trans-generational effects). Whereas direct effects of the environmental conditions on immune defence are well-studied in many organisms, the understanding of predictive adaptive responses, such as trans-generational effects, are still scarce, especially in natural populations. One hypothesis is that mothers stressed during development produce offspring that are able to cope in a better way with similar type of stress during their own development in comparison to control individuals. In this poster presentation, I will show preliminary results from an experiment in which we assessed possible immunological maternal effects in the Glanville fritillary butterfly (*Melitaea cinxia*).

POSTER SESSION: Evolutionary phylogenetics

POSTER 103.

Molecular phylogeny of the genus *Yphthimoides* (Satyrinae: Euptychiina)

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The subtribe Euptychiina (Satyrinae) is overwhelmed by non-natural genera and countless undescribed species, and several studies have pointed the necessity of additional taxonomic and phylogenetic studies to better understand the limits among the genera and species of this group. One of these genera, *Yphthimoides* (Forster, 1964), has been shown to be an unnatural assemblage based on morphological and molecular evidences. In the present study, preliminary analysis of Bayesian Inference using the first part (˜650bp) of the mitochondrial gene Citochrome C Oxidase subunit I (Cox I) corroborate the hypothesis that *Yphthimoides* is not monophyletic, with *Yphthimoides affinis*, *Yphthimoides maepius* and *Yphthimoides mimula*, belonging to another clade. Present results also revealed the existence of four new undescribed species of *Yphthimoides*, based on both, molecular and morphological (wing pattern and morphology of the male genitalia) evidences, and suggested that *Yphthimoides renata* could include at least two cryptic species (*Y. renata* from Central America is sister to *Yphthimoides leguialimai* and not to the South American *Y. renata*). With the inclusion of nuclear genes in these analyses, a better resolution would be attained, leading to a better understanding of the relationships among the species in the genus *Yphthimoides*.

POSTER 104.

Temporal hybrid dynamics in the *Papilio machaon* species group in western Canada

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Six decades ago, Sir Cyril Clarke and Philip Sheppard stated that the *Papilio machaon* group of swallowtail butterflies "provides some of the most suitable material ever investigated. . . for studying the process of speciation in detail" and highlighted the need to integrate genetic, ecological, and behavioral data within a thorough understanding of evolutionary time. The dynamics of evolutionary timescales influence every aspect of speciation, and increasing anthropogenic influences in recent times amplify the importance of understanding these timescales. However, comparable historical and modern studies that address the impact of even short evolutionary timescales are rare. Here we compare historical (˜30 years ago: morphometric, allozyme) and recent datasets (morphometric, DNA sequence, microsatellites, SNPs) to characterize dynamics within a region of potential hybrid interaction between two species of the *P. machaon* complex, a group with a large proportion of its biodiversity resulting from ancient hybridization. Although the two species are found across Alberta and British Columbia, and produce viable hybrid offspring in lab crosses, both surveys found that hybrid individuals were mainly restricted to a few central regions. The ancestry of these hybrids varied, with allozymes producing distinct patterns of early generation hybrids (F1s) that were not observed in recent genetic data. However, heterozygosity and hybrid-index measures did not differ significantly between datasets, suggesting that the composition of hybrid populations is relatively stable, despite complexity at the individual level. This stability, albeit through a short timescale, provides valuable insight into a group where many hybrid lineages seem to have progressed to being distinct evolutionary entities. Such use of "historical" datasets to evaluate the temporal dynamics of speciation will only become more common in future years, and the *P. machaon* group, as Clarke and Sheppard predicted, will surely continue to serve as a valuable model system.

POSTER 105.

Anomalous areas and butterflies – phylogeny of *Leptotes* (Lepidoptera: Lycaenidae)

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About 30 species of blues of the genus *Leptotes* (Lepidoptera: Lycaenidae) are known from tropical and subtropical areas around the globe. In every region, only few species have very wide distribution, about one third of the taxa are endemic to islands and several species are endemic to Andes. Only Africa deviates from this pattern as it has five species of *Leptotes* sympatric in large areas over the continent. Working on molecular analyses using two mitochondrial and two nuclear genes, we have found that the genus Leptotes is paraphyletic with *Cyclyrius*. The oldest branch is formed by Indo-Australian species *Leptotes plinius*. Species radiation was observed in Neotropic and Afrotropical regions. The distribution pattern is very old and correspond with species evolution, no recent shift between continents was found. European populations of *Leptotes pirithous* do not differ from populations from Africa and similarly Nearctic species were derived from Neotropic species. The study was supported by Grant Agency of the Czech Republic (14-36098G) and by University of South Bohemia (168/2013/P).

POSTER 106.

Cryptic diversity in *Driopa* (Lepidoptera: Papilionidae: Parnassiinae) butterflies

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Morphological, ecological and molecular evidence of existence of cryptic species in *Driopa* (Korshunov 1988) subgenus are analysed. *Driopa* includes species whose larvae feed on plants of the genus *Corydalis* (Fumariaceae): *Parnassius mnemosyne*, *P. nordmanni*, *P. clodius*, *P. eversmanni*, *P. glacialis*, *P. stubbendorfii* and *P. ariadne*. All of these species have a Central-Eastern Asian distribution, except for *P. clodius*, which occurs in Western North America, *P. nordmanni*, restricted to the Caucasian Range, and *P. mnemosyne*, which inhabit a large area from Central Asia to Western Europe. We used a large set of sequences (our and from GenBank) of mitochondrial COI gene to reconstruct the subgenus phylogeny and history across about a million years. BEAST 1.8.0 was used for calculations of the time to tMRCA and obtaining phylogenetic tree; general mixed Yule- coalescent model was applied then to estimate species delimitation threshold and cryptic diversity. In result we obtained a tree with root at 0.802 Ma with relatively wide 95% HPD interval at 0.5041-1.1329. The single-threshold GMYC show us a huge diversity inside Driopa, inferring 25 different species that in nearly 2.5 times more than is delineated using only morphological features. We also performed species distribution modelling for two deeply divergent COI clades of P. mnemosyne using MAXENT 3.3.3. These two large clades were defined also by the previous studies of phylogeny of Parnassian butterflies (Gratton 2006, 2008; Michel et al. 2008), the first one occurs in the West Europe, to the South from the Alps (Spain, France, and Italy, “WM-group”) and the second one inhabits the large areas across Central, Eastern Europe and Central Asia (“AE-group”). Our SDM analyses show large differences in predicted distributions and in ecological preferences of these groups. Also we found some differences in male and female genitalia between *P. mnemosyne* specimens from Pyrenees and Eastern Europe.

POSTER 107.

Diversification and biogeography of the *Papilio* (*Menelaides*) swallowtail butterflies of the Indo-Australian Region

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Drivers of speciation and diversification remain poorly explored in highly biodiverse tropical regions. One such tropical region that has been very important since Wallace is the Indo-Australian Region. Topographical, climatic and ecological heterogeneity, and biogeographic complexity, have arguably influenced diversity, distribution and endemism in this region. Here, we aim to study the influence of geography and ecology in the evolution of biodiversity in this region using a striking adaptive radiation of *Papilio* (*Menelaides*) swallowtail butterflies. *Menelaides* has ca 50 species and 200 subspecies, and it is also morphologically diverse with widespread Batesian mimicry that manifests in sex-specific and polymorphic forms. To study this species and morphological radiation, we have generated a species-level molecular phylogeny of *Menelaides* based on multiple subspecies (˜200 individuals) and six nuclear and mitochondrial markers (˜4.2kb). We used Bayesian and Likelihood approaches to reconstruct phylogenetic relationships, and delineated *Menelaides* as a monophyletic group by revising subgenus assignment of the demolion-antonio-noblei species group. Further, we delineated species within *Menelaides* using a multi-gene sequence dataset and coalescent approach in Bayesian framework (*BEAST, BEST and bPTP). This analysis showed that nine biogeographically isolated and morphologically very distinct species are embedded within larger *memnon* and *aegeus* species groups, suggesting complex speciation patterns in this zoogeographic region. We also identified ten clades of what are currently considered to be subspecies of *polytes*, *helenus* and *fuscus*, which are potentially distinct species that have diversified considerably in allopatry on isolated island groups. On the whole, *Menelaides* radiation has taken place during the Miocene for 145 mya, with dispersal and allopatry being the primary drivers of speciation. We will now integrate biogeographic and speciation analyses with wing pattern evolution to understand relative roles of biogeographic processes and ecological interactions (i.e., mimicry) in driving speciation and diversification of the Indo-Australian *Papilio* (*Menelaides*) swallowtails.

POSTER 108.

Tracing the radiation of *Maniola* (Lepidoptera: Nymphalidae: Satyrinae) butterflies: new insights from phylogeography hint at one single incompletely differentiated species complex

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Maniola butterflies show a peculiar distribution: *Maniola jurtina*, the most widely distributed species of this genus, occurs all over Europe, whereas the other six described species are restricted to the Mediterranean area, among them three are island endemics on Sardinia, Cyprus and Chios, respectively. The species are almost indistinguishable morphologically and hybridize if sympatric. To clarify species boundaries and the diversification history of the genus, we reconstructed its phylogeny using the genetic markers COI, CytB, Elongation factor 1a and wingless, analysed variation in mitochondrial and nuclear DNA, estimated divergence times, compared possible migration routes and inferred demographic developments from genetic models. The topology of the recovered phylogenetic tree is not consistent with accepted taxonomy, but rather reveals several haplotype clades that are incongruent with nominal species boundaries. This represents a rare case where several taxa earlier described by means of morphological traits become merged into fewer species (or even a single one) when using genetic markers. Usually more (cryptic) species are uncovered with genetic methods than have been taxonomically described using morphological characters. Instead of seven species, I recognized two major lineages which could have formed during an out-of-Africa scenario: one part of the *Maniola* genus migrating over the Strait of Gibraltar and the Iberian Peninsula to the west of Europe, and the other part wandering eastwards through Asia Minor and over the Bosporus into eastern Europe. Genetic diversity proved to be surprisingly high, even in island species. Furthermore, molecular data suggest that *Maniola* is a very young taxon (about 0.6 to 0.9 Mya old).

POSTER 109.

Out of Africa and back again: the biogeography of *Melanitis* butterflies (Nymphalidae: Satyrinae)

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The genus *Melanitis* comprises 2 African species, 9 Asian species, and a single species, *M. leda*, found throughout the Old World. We sequenced six molecular markers from ˜70 specimens representing all *Melanitis* species and close relatives *Gnophodes* (African) and *Bletagona* (Sulawesi endemic). We included as outgroups representatives of all other genera in the tribe Melanitini (including the first DNA sequences from the Indian genus *Parantirrhoea*) and rooted the tree with an *Elymnias* species. A species tree analysis of the group demonstrates that *Melanitis* originated in Africa, dispersed to Asia where it diversified, and the single species *M. leda* originated in Asia and dispersed back to Africa. The genera *Gnophodes* and *Bletagona* are both apparently embedded within *Melanitis*, suggesting that taxonomic revision is warranted.

POSTER 110.

Who's the Monarch's sister? Gene conflict in the phylogeny of *Danaus* butterflies (Nymphalidae: Danainae)

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*Danaus*butterflies are conspicuous members of every tropical butterfly fauna, and *D. plexippus*in its ancestral North American range famously undergoes a unique, multi-generational migration. Previous phylogenetic investigations of *Danaus* have not thoroughly sampled species, characters, or both. As a result, the sister species of the *D. plexippus* is unclear and the evolutionary history of the group is ambiguous. We sampled 150 *Danaus* specimens including all recognized and putative species, and sequenced 4 – 7 molecular markers to examine the biogeographic and evolutionary history of the group. Nuclear and mitochondrial markers infer strongly supported but different evolutionary histories of the group. Notably, mitochondrial genes implicate the Caribbean endemic *D. cleophile* as the sister to *D. plexippus*, while nuclear genes usually infer the Southern Monarch, *D. erippus*. Mitochondrial data suggest that *D. affinis*, *D. melanippus*, and *D. genutia* should each be split into two or more species, while nuclear data supports their monophyly. Hybridization or incomplete lineage sorting might be responsible for these conflicting results. We find no evidence of systematic Wolbachia infection that could lead to this mito-nuclear discord. Our data support the separation of *D. plexaure* and *D. petilia* as distinct species, but not *D. dorippus*. *Danaus* might be a model system for the study of reticulate evolution in animals, in addition to invertebrate migration.

POSTER 111.

The phylogeny of *Stalachtis* (Riodinidae) and considerations on mimicry patterns evolution

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With seven recognized species, the south-american *Stalachtis* is the sole genus currently treated in the tribe Stalachtini, within the family Riodinidae. *Stalachtis* butterflies present aposematic patterns, and are clearly part of mimetic rings including ithomiines, heliconiines and other Lepidoptera. The main objective of the present study is to obtain a phylogenetic hypothesis for the genus based on molecular markers and, based on the obtained topology, understand the evolution of color pattern within the genus. A total of 79 specimens were sampled, comprising six out the seven described species for *Stalachtis*. The total genomic DNA was isolated from 60 individuals, and three gene regions were sequenced: cytochrome c oxidase subunit I (COI), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and ribosomal protein S5 (RpS5). Bayesian analysis was carried out for the concatenated data set under the GTR+model, using *Styx infernalis* as outgroup. The analysis recovered *Stalachtis* and all its species monophyletic, with high support for all clades. The widespread *Stalachtis phlegia* clade was the sister group of all other species, and present data support the revalidation of *Stalachtis susanna* as a good species (and not a subspecies of *S. phlegia*). Mimetic patterns are stable within clades, with the striped pattern of *S. lineata*, *S. euterpe* and *S. phaedusa*, probably arising in the ancestor of all other *Stalachtis* species, except the *phlegia* clade. The tiger orange pattern, present only in the *S. calliope* + *S. magdalena* clade, is possibly the most recent mimetic pattern in the group.

POSTER 112.

Mark-recapture and genetic estimates of population size in a protected moth

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Population size is the keystone of many decision-making models in population genetics and ecology. Estimates of long-term and contemporary effective population sizes (Ne), as well as of census population size (N) are crucial for the efficient management of endangered species. However, the spatial and temporal stratification of wild populations make these measures difficult to interpret. Here, we estimate these parameters for the protected moth *Graellsia isabellae* in two contrasting areas, Puebla (Eastern Iberia-EI) (n = 61) and Ordesa Valley (Western Pyrenees-WP) (n = 134), corresponding, respectively, to an isolated unstructured population, and to a set of localities interconnected by pine woodlands that should facilitate gene flow among them (metapopulation). According to our phylogeographic analyses of mitochondrial haplotypes, the Pyrenees are most likely to have been re-colonized by *G. isabellae* after the LGM by individuals from EI, following the dispersion of their host plant, Pinus sylvestris, from this glacial refugium to the north. In agreement with this biogeographic hypothesis, the mismatch distribution of mitochondrial haplotypes in WP shows unequivocal footprints of a population expansion, and long-term Ne estimates based on microsatellite nuclear loci are three times higher for EI than for WP. Referring to a more recent past, molecular signatures of microsatellites at WP are consistent with population bottlenecks. If considering each of the WP localities separately, estimates of contemporary Ne are also much lower for WP than for EI, but not quite so when the analysis is carried out for the WP metapopulation as a whole. Finally, MRR experiments indicate that the density of *G. isabellae* might be 50% higher in EI than in WP, and draw a pattern of pulses in the window of emergence shown by the adults, which may add up to the structuring of reproduction in each generation, with the concomitant decrease in contemporary Ne.

POSTER 113.

DNA-barcoding survey revealed necessity of taxonomic revision of Israeli butterflies (Lepidoptera, Papilionoidea)

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Species are important practical units in evolution, ecology and conservation. However, every species list contains uncertainties due to (1) the evolutionary continuum between higher taxa, species and populations, (2) imperfect taxonomy, and (3) different species concepts and taxonomic cultures (particularly “splitters”versus “lumpers”). These three types of uncertainty can never be eliminated completely. However, species lists can be made much more useful if ambiguities are minimized or resolved (Isaak et al. 2004). In our research, we used DNA-barcoding approach to reveal uncertainties of the second and third kind in the butterfly species list of Israel. We obtained DNA- barcodes for 70 species representing approximately 50% of Israeli butterfly fauna, and we are working to complete barcode coverage for all butterfly species of Israel in the near future. Butterflies represent the best taxonomically studied group of invertebrate animals, and Israel is one of the best studied territories in the world with respect to butterfly fauna. Despite these facts, the DNA-barcoding survey revealed a number of serious conflicts between traditionally accepted taxonomic interpretations of Israeli butterfly populations and patterns of genetic diversity revealed by DNA-barcodes. We consider these discrepancies as signal flags for a deeper taxonomic analysis of these populations by using morphological, chromosomal and multiple molecular markers.

POSTER 114.

Development and characterization of microsatellite loci in the White *Morpho* butterflies (Lepidoptera: Nymphalidae), from Brazilian Atlantic Forest

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The white *Morpho* from South America form a complex of several highly similar taxa. Traditional morphological studies recognized two species and six subspecies within this complex, but the validity of these taxa remains uncertain, and changes depending on the author. Studies of genetic structure, gene flow and outcrossing rate may assist in solving taxonomic and ecological questions, and also contribute to conservation. For this, the microsatellite markers (SSR) are considered ideal because they are codominant and highly informative. The development of SSR primers specific for the species constitutes one of the first steps in such studies. The purpose of the present study was to develop microsatellite markers, aiming to elucidate the taxonomic and ecological questions related to the white Morpho species complex. A genomic microsatellite-enriched library was developed for a population from coastal Rio de Janeiro, Brazil (= *Morpho epistrophus*). Forty-one from 96 sequences analyzed contained SSRs motifs, representing a 42.70% rate of enrichment success. Di-nucleotide repeats were the most abundant class of microsatellites (68.30%), followed by tetra-nucleotide (24.40%) and tri-nucleotide repeats (2.45%). Most of the identified SSRs were classified as perfect (95%). In a second classification, 73.17 % were Class I (20 bp) and 26.83 % were Class II (12 to 20 bp). In total, 25 specific primers pairs were designed, 17 amplified successfully in white *Morpho* and 12 SSRs were polymorphic, which allowed the identification of 3 to 19 alleles per locus from the analysis of 30 individuals. These polymorphic markers should provide efficient tools to the study of the population genetic structure of these butterflies.

POSTER 115.

Making phylogenetically comprehensible the hyperdiverse Polyommatina butterflies

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The challenge of making phylogenetically comprehensible a major living group relies on the availability to compile representatives for the entire diversity and the capability to accommodate the resulting relationships to pre-existing nomenclature. Here we present molecular phylogenetic surveys throughout the subtribe Polyommatina (Lycaenidae, Polyommatinae), representing ca 460 species. We perform a first comprehensive phylogenetic reconstruction including the 81 described genera and nine markers. A full taxonomic revision based on a flexible temporal scheme results in the retention of 31 genera, plus the description of one new genus. In an extension of the dataset, a 1100-specimens phylogenetic tree is gathered, comprising about 80% of the known specific taxa. We evaluate the approach of combining high proportions of specimens with single markers on a multilocus-based phylogenetic framework, and we show it to be a powerful tool for taxonomic improvements, allowing to place uncertain taxa into genera and discovering intriguing deep divergent lineages. Overall, we deeply investigate the phylogenetic history of the hyperdiverse Polyommatina butterflies, a first necessary step for any further accurate evolutionary research.

POSTER 116.

Cryptic Diversification of Ornamental Butterflies across Tropical Biodiversity Hotspots

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Tropical hotspots are astonishingly diverse yet anthropogenically threatened, however most of their species richness remains unknown. Recently a plea has been made to accelerate species discovery using molecular species delimitation techniques coupled with parallel lines of other evidence in an integrative approach. Cryptic species complexes may thus be revealed in a thorough fashion where traditional taxonomy might have limits. Here, we unveil cryptic diversification in the highly emblematic tropical Nawab butterflies across ten biodiversity hotspots of the Indomalayan / Australasian archipelago. Combining multimarker molecular phylogenetics and different species delineation methods we discover unexpectedly high levels of cryptic differentiation. We discover two new species, and six populations are raised to species status increasing diversity by more than 25%. These species are surprisingly not only island endemics but also independently evolving continental lineages. Our findings strongly advocate the use of integrative approaches to unveil patterns of species richness and catalogue Earth's concealed, therefore unappreciated diversity.

POSTER 117.

‘Bad’ species? RADsequencing, DNA barcoding, and a role for *Wolbachia* in the evolutionary history of the *Erebia tyndarus* species complex

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The *Erebia tyndarus* species complex is a cluster of closely related alpine butterflies. Members of this group have been characterized so far by morphological and ecological characters, cross-breeding experiments, karyology, and molecular data from allozymes and mitochondrial DNA (mtDNA). However, the delimitation of species, their relationships, as well as the origin of their peculiar geographic distribution, are still largely unclear. We employed restriction sites associated DNA (RAD) sequencing in order to clarify the systematic and genetic relationships among taxa of the *E. tyndarus* “Alpine” clade. Our results showed, for the first time, that the four species traditionally recognized in this clade (*E. tyndarus*, *E. calcaria*, *E. nivalis*, *E. cassioides*) indeed correspond to very well defined genetic units, which can be easily identified even by analysing small subsets of 15-20 of polymorphic markers. A species tree analysis based on a subset of 400 SNPs indicate that the four species gradually diverged from each other within the last 150,000 years. In contrast to the neat delimitation provided by nuclear RAD data, we did not find any species-specific synapomorphies at 2100 bp of mtDNA (Figure 1b), despite a clear intraspecific geographic structure. Moreover, our RADsequencing approach allowed to reveal the presence of *Wolbachia* endosymbionts in all of the species and geographic regions. We thus used coalescent simulations to compare the probability of the observed mtDNA data under a ‘neutral’ scenario of divergence fitted to our RAD-SNPs data and under a non-neutral scenario involving mtDNA replacement driven by Wolbachia infection. Results clearly indicate that a neutral scenario is extremely unlikely, and that the lack of interspecific mitochondrial differentiation in the *E. tyndarus* ‘Alpine’ clade is most probably due to a *Wolbachia*-driven mitochondrial replacement occurred ca. 15,000 – 40,000 years BP.

POSTER 118.

Cytogenetic mechanisms and evolutionary dynamics of chromosome number evolution in butterflies (Insecta: Lepidoptera)

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The order Lepidoptera is generally characterized by a stability of karyotypes, and between-species variation in chromosome number is usually very low or absent. Most species have the same haploid number of chromosomes, n = 30 or 31. However, several complexes of closely related species of butterflies demonstrate high level of chromosome number variation. For instance, the subgenus *Polyommatus* (*Agrodiaetus*) of the family Lycaenidae is characterized by one of the highest variation in chromosome numbers across invertebrates (from n=10 to n=134). The variability is not associated with polyploidy and is caused by multiple chromosome fusions and fissions. Chromosome stability, on one hand, and high variability in the chromosome numbers, on the other hand, suggest that in the evolution of butterflies periods of chromosome conservatism can be interrupted by periods of fast karyotype changes. In our research we addressed the questions of cytological and evolutionary mechanisms underlying the rapid change of chromosome number. First, using methods of classic and molecular cytogenetics (such as fluorescent in situ hybridization), we conducted a comparative study of karyotypes in groups with a high level of interspecific karyotype diversity. A special attention was paid to *Polyommatus* (*Agrodiaetus*) phyllis species group, in which closely related or even sister species were discovered to have extremely different chromosome numbers. Second, we applied comparative phylogenetic methods to check different hypotheses about rapid chromosomal evolution. It has been tested whether chromosomal numbers possess a phylogenetic signal. We also checked the compliance of karyotype evolutionary dynamics to the models of complex trait evolution. Based on phylogenetic data and distribution of traits across the phylogenetic tree, our approach allows defining rapid karyotype evolution in terms of Brownian motion model. Thus, karyotype diversity evolutionary dynamics can be explained by a stochastic process without implication of natural selection.

POSTER 119.

Hidden diversity of Caucasian blues of the *Polyommatus* (*Agrodiaetus*) *ripartii* species complex (Lepidoptera, Lycaenidae): Assessing species taxa by chromosomal and molecular data

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The butterflies of the Western Palearctic *Polyommatus* (*Agrodiaetus*) *ripartii* species complex are model systems in evolutionary research, and are important in conservation. Despite this fact, their taxonomy is poorly elaborated since

the group mostly consists of morphologically undifferentiated species. *P. valiabadi* sensu auctorum is supposed to be the only exception from this rule due to accurately distinguishable pattern of the wing underside. In our work we used a combination of morphological, chromosomal and molecular markers to perform a taxonomic analysis of all described species belonging to the *P. ripartii* complex. We discovered that in fact *P. valiabadi* sensu aucrotum is a triplet of cryptic species, strongly differentiated by their karyotypes and mitochondrial haplotypes. Two species are found to inhabit in sympatry in South Azerbaijan, the third species is an endemic of Elburs Mountains in Iran. Funding: The work was supported by the Russian Foundation for Basic Research (grants 12-04-00490 and 14-04-31726). A.Dantchenko was supported by the grant 1.50.1617.2013 of the Saint-Petersburg State University, Russia.

POSTER 120.

Morphological phylogeny of the Batesian-mimicking butterfly genus *Elymnias* Hübner, 1818 (Lepidoptera, Nymphalidae, Satyrinae)

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The genus *Elymnias* Hübner, 1818 is one of the most species-rich groups of butterflies (48 species and 201 subspecies), and occurs throughout Southeast Asia plus two species in Africa. All the species vary in wing shape and color pattern due to the diversified Batesian mimicry relationships with various lepidopteran models. Examination of museum specimens suggests that at least ten types of wing patterns are recognized for the purpose of discussion of crypsis, aposematism and mimicry. Some *Elymnias* species show dramatic variation in wing patterns among individuals or subspecies, presumably to mimic models that are similarly variable in different localities. This variability, coupled with genitalic conservativeness, complicates species delimitation as well as phylogenetic inference. The aims of this study were to address several key questions: 1) Are similar mimetic color pattern evolved convergently or conserved phylogenetically? 2) Has sexually dimorphic mimicry evolved multiple times? 3) Did the *Elymnias* lineage evolve in Africa or Southeast Asia? We reconstructed the phylogeny based on 142 adult morphological characters (including 132 from wing pattern) obtained from 39 species using both equal weighting (EW) and implied weighting (IW) under maximum parsimony criterion. Character were optimized using WInclada 1.00.08. The results show both genitalic and wing pattern characters are highly homoplasious, and most nodes are weakly supported due to convergence and conservativeness. However, wing pattern characters are not necessarily more homoplasious than genitalia even evolved in mimicry. All the mimetic wing pattern types and sexually dimorphic mimicry are obviously evolved independently. The fact that the African species always grouped within the Asian lineages suggests the genus originated from Asia.

POSTER 121.

Cryptic species of *Perisama* (Nymphalidae, Biblidinae) from the Cordillera de Merida in Venezuela

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The genus *Perisama* Doubleday [1849] includes currently 42 species and over 80 subspecies. It occurs almost exclusively in the tropical Andes, except for one species from known the Guyana Shield in SE Venezuela. *Perisama oppelii* (Latreille, [1809]) is widespread and highly polytypic, with 9 subspecies whose taxonomy relies on wing colour pattern differences and geographical distribution, rarely with any reference to genital morphology. We examined over 270 specimens of all the subspecies of *Perisama oppelii* and made 20 genital dissections. A comparative analysis shows very slight intraspecific variation in male genitalia of *Perisama oppelii*, with the notable exception of *P. oppelii bleuzeni* Attal & Crosson du Cormier from the Venezuelan Cordillera de Merida. In particular, the shape of hypandrium of this subspecies stands apart not only from other subspecies of *P. oppelii* but also differs significantly from other species of *Perisama*. Hypandrium, the modified eighth sternum, determines the position of genitalia during mating, and therefore may be an important pre-zygotic isolation factor. Hence, its shape and structure can be considered as a particularly valuable taxonomic character. Based on this assumption, *P. oppelii* should be considered as a separate species. This study further emphasizes the role of genital characters in resolving species-level taxonomy of Biblidinae.

POSTER SESSION: Behavioural ecology

POSTER 122.

The brain of *Godyris zavaleta*, an olfactorily driven butterfly: general layout and sexual dimorphism.

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Comparisons of brain atlases can be highly informative, enhancing our understanding of the structure and function of distinct neuropil, identifying potential adaptations to species-specific ecologies, and examining the malleability of neurological connectivity and the extent of co-evolution between related structures. The value of interspecific comparisons are greatly enhanced when the species considered have known ecological differences. The Lepidoptera have long been a favoured model in both neurobiology and evolutionary biology, but brain atlases have largely focused on a small number of species. Here we describe the brain of *Godyris zavaleta* (Ithomiinae), a member of a tribe of neotropical butterflies with enhanced reliance on olfactory information. We demonstrate the presence of sexually dimorphic glomeruli within a distinct macrogomerular complex in the antennal lobe for the first time in diurnal butterflies. Through an interspecific analysis across four Lepidoptera we further show that sensory adaptations between species closely mirror their activity patterns and sensory preference, with *G. zavaleta* displaying a level of investment in visual and olfactory neuropil intermediate between the diurnal, visually driven Monarch butterfly (*D. plexippus*) and night-flying, olfactorily driven moths. Finally, we identify a number of anatomical traits that distinguish the two butterflies from moths, and several that distinguish *D. plexippus* from *G. zavaleta*. Our results illustrate the potential for selection to mould the structure of invertebrate brains, and furthermore, the potential for comparative analyses across ecologically divergent species to serve as a tool for understanding the functional significance of variation in brain structure, and the nature of connections between brain components.

POSTER 123.

Deimatic display in the European swallowtail butterfly as a secondary defence against attacks from great tits

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Many animals reduce the risk of being attacked by a predator through crypsis, masquerade or, alternatively, by advertising unprofitability by means of aposematic signalling. Behavioural attributes in prey employed after discovery, however, signify the importance of also having an effective secondary defence if a predator uncovers, or is immune to, the prey's primary defence. In butterflies, as in most animals, secondary defence generally consists of escape flights. However, some butterfly species have evolved other means of secondary defence such as deimatic displays/startle displays. The European swallowtail, *Papilio machaon*, employs what appears to be a startle display by exposing its brightly coloured dorsal wing surface upon disturbance and, if the disturbance continues, by intermittently protracting and relaxing its wing muscles generating a jerky motion of the wings. This display appears directed towards predators but whether it is effective in intimidating predators so that they refrain from attacks has never been tested experimentally. In this study we staged encounters between a passerine predator, the great tit, *Parus major*, and live and dead swallowtail butterflies in a two-choice experiment. Results showed that the dead butterfly was virtually always attacked before the live butterfly, and that it took four times longer before a bird attacked the live butterfly. When the live butterfly was approached by a bird this generally elicited the butterfly's startle display, which usually caused the approaching bird to flee. We also performed a palatability test of the butterflies and results show that the great tits seemed to find them palatable. We conclude that the swallowtail's startle display of conspicuous coloration and jerky movements is an efficient secondary defence against small passerines.

POSTER 124.

Using optical properties of wing metallic sheen to evaluate the mimicry relationships between *Elymnias hypermnestra* and the sympatric *Euploea* species

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The co-mimics of the unpalatable *Euploea* butterflies include various palatable taxa, such as the satyrine genus *Elymnias*. In Taiwan and southern China, *Elymnias hypermnestra hainana* is often considered as the mimic of various "blue *Euploea*" species. The visual resemblance between them is not only contributed by wing morphology and behavior, but also by the metallic blue sheen produced by interactions between the optical properties and microstructures of wing scales. In mimicry biology, it is essential to determine the model-mimic relationship between co-mimicking species, but it becomes challenging when multiple unpalatable species with similar colouration and chemical defenses are sympatric with one mimic. Apart from assessing the relative palatability, phenology, and degree of sympatry between the potential model and mimic, the accuracy of wing pattern match (visual signal) is one of the most important factors in determining a mimicry relationship. Most case studies of defensive mimicry focus on the visual signals irrelevant with metallic sheen, so there is no need to measure the optical properties. In the present study, we attempted to evaluate the similarity of the metallic sheen between the Taiwanese *E. hypermnestra hainana* and 3 species of sympatric *Euploea* by measuring the reflectance spectra using a spectrophotometer (OceanOptics usb4000). The results show that the reflectance spectra of them were similar and peaked around 500nm. However, reflectance intensity of *E. hypermnestra hainana* was relatively weak as compared with the *Euploea* species. We suggest that the dis-similarity caused by the slight differences in optical properties may be compensated by inhibiting different microhabitats. Further study on the evolutionary origin of the three *Euploea* species as well as the role of the imperfectly mimicking male *E. hypermnestra hainana* are needed in order to confirm how the predators perceive the signals between three models and one sexually dimorphic mimic.

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