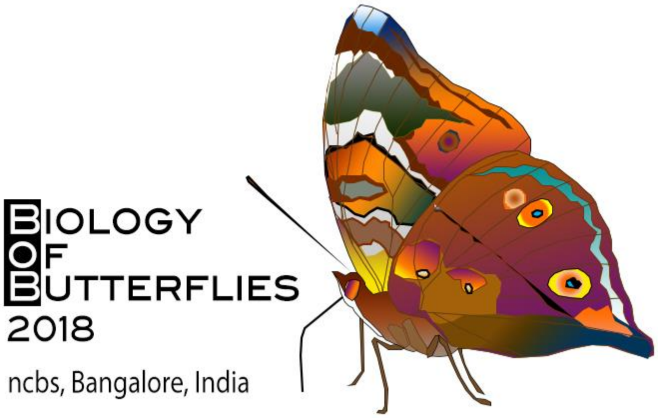
8th International Conference on the Biology of Butterflies

11-14 June 2018



Abstract Book

ncbs

IISER
THIRUVANANTHAPURAM

PREFACE

Welcome to the 8th International Conference on the Biology of Butterflies (ICBB) at the National Centre for Biological Sciences (NCBS), Bangalore, India, from 11-14 June 2018. The ICBBs are international gatherings, organized every four years, of primarily professional biologists who study evolutionary biology, behaviour, ecology, systematics, biogeography, genetics, developmental biology, and biodiversity conservation, with butterflies and moths as their focal study organisms. This is an excellent academic and professional forum for latest discoveries and to exchange news in these research areas. The 8th ICBB is being organized by NCBS and the Indian Institute of Science Education and Research (IISER) Thiruvananthapuram, and this is first time an ICBB is being held in a tropical country.

We hope you will have a wonderful time at the conference! Should you have any queries regarding logistics, please contact one of the volunteers or organizers.

Krushnamegh Kunte and Ullasa Kodandaramaiah,Organizers.

Monday 11th June	**Tuesday 12th June**		**Wednesday 13th June**	**Thursday 14th June**	
Symposium: **Interacting Butterflies: From Genes to Communities**	Symposium: **Pattern formation and Evo-Devo in Butterflies**	Symposium: **Ecological Immunology in Butterflies**	Symposium: **Evolution and Diversification of Aposematism and Mimicry Systems**	Symposium: **Diversification, Speciation and Biogeography in the Tropics**	Symposium: **Foraging Ecology of Butterflies**
Venue **Dasheri**	**Venue** **Dasheri**	**Venue** **LH1/Haapus**	**Venue** **Dasheri**	**Venue** **Dasheri**	**Venue** **LH1/Haapus**
08:50 **Cultural Dance**					
09:00 **Plenary**	**9:00** **Plenary**		**9:00** **Plenary**	**9:00** **Plenary**	
09:45 Symposia Talks	09:45 Symposia Talks	9:45 Symposia Talks	9:45 Symposia Talks	9:45 Symposia Talks	9:45 Symposia Talks
11:00 **Tea**	**11: 00** **Tea**		**11:00** **Tea**	**11:00** **Tea**	
11:20 Symposia Talks	11:20 Symposia Talks	11:20 Symposia Talks	11:20 Symposia Talks	11:20 Symposia Talks	Symposium: **Sensory Ecology of Butterflies and Moths** 11:20 Symposia Talks 12:35 Lunch
12:35 Lunch	12: 35 Lunch		12: 35 Lunch	12: 35 Lunch	
	14:00-14:15 Symposium Talk			14:00-15:30 Symposia Talks	
Symposium: **Impact of weather trends and extremes on long-term population dynamics**	Symposium: **Colourful Stories: The How's and the Why's of Butterfly Structural Colours**		Symposium: **Notes from the Field : Observations and Hypotheses**		
14:00 **Plenary**	14:15 **Plenary**		**14:00** **Plenary**		
14:45 Symposia Talks	15:00 Symposia Talks		14:45 Symposia Talks		
16:00 **Tea**	**15:45** **Tea**		**16:00** **Tea**	**15:30** **Tea** **Symposium:** **Global Citizen Science Monitoring of Butterflies: Building a Globally Engaged Community of Science, Education and Conservation**	
16:20 Symposia Talks	16:05 **Open Symposium**		16:20 Symposia Talks	**15:50** **Plenary Talk**	
17:15 Beauty of Butterflies	**17:30** **Posters**		**17:15** **Posters**	**16:35** Symposia Talks	
18:45 **Dinner**	**18:45** **Dinner**		**18:45** **Dinner**	**18:45** **Banquet**	

Schedule:

Monday, 11th June 2018

Interacting Butterflies: From Genes to Communities

Organizers	**Robert Tropek and Marianne Espeland**	**Abstract**
Plenary	**Naomi Pierce**	Page 14
Title	Ecological specialization in the Lycaenidae and its role in diversification	
09:00	Dasheri	

Venue		**Dasheri**	
Time	**Speaker**	**Talk Title**	**Abstract**
09:45	**Niklas Janz**	Tempo and mode of butterfly host-driven diversification	Page 19
10:00	**Matthew Forister**	Understanding persistence on a novel host in the context of phytochemistry, microbes and arthropod communities	Page 19
10:15	**Christopher Wheat**	Genetic basis of a female-limited alternative life history switch & insights into the biotic interactions maintaining it within populations	Page 20
10:30	**Robert Tropek**	Role of butterflies in pollination networks of Mount Cameroon	Page 20
10:45	**Ranjit Sahoo**	Host plant abundance explains negative association between larval performance and female preference	Page 21
11:00 – 11: 20		**Tea break**	
11:20	**Kruttika Phalnikar**	Butterfly male killers: Intracellular bacterium induces female biased sex ratio in a butterfly	Page 21
11:35	**David Lohman**	A geographic mosaic of *Wolbachia* infection in *Melanitis leda* butterfly populations	Page 22
11:50	**Deepa Agashe**	The missing association between butterflies and their gut bacteria	Page 23
12:05	**Marianne Espeland**	The impact of mutualistic and parasitic life histories on butterfly diversification in an increasingly arid world	Page 23

Monday, 11th June 2018

Impact of weather trends and extremes on long-term population dynamics

Organizers	**Jens Roland and Steve Matter**	**Abstract**
Plenary	**Marjo Saastamoinen**	Page 14
Title	Increased synchrony in weather conditions drives increased synchrony in metapopulation dynamics of the Glanville Fritillary butterfly in Finland	
14:00	Dasheri	

Venue		**Dasheri**	
Time	**Speaker**	**Talk Title**	**Abstract**
14: 45	**James Fordyce**	Butterflies and climate: regional declines, local responses, and increasing unpredictability	Page 24
15: 00	**Erica Henry**	The effects of temperature and precipitation on population dynamics of both common and endangered butterflies	Page 24
15: 15	**Jens Roland**	Early-winter extreme weather dominates dynamics of alpine *Parnassius smintheus* butterflies in the Rocky Mountains of Canada	Page 25
15: 30	**Stephen Matter**	Let it snow, let it snow, but not too much: Overwintering survival affects population growth of *Parnassius smintheus*	Page 25
15: 45	**Robert Srygley**	El-niño, hostplant growth and migratory butterfly abundance	Page 26
16:00 – 16: 20		**Tea break**	
16: 20	**Michelle Davis**	Conservation genetics of the Marsh Fritillary butterfly *Euphydryas aurinia* in Great Britain	Page 26
16: 35	**Matthew Nielsen**	Misinformation in a new climate: Photoperiod-induced seasonal polyphenism under climate change	Page 27
16: 50	**Timothy Bonebrake**	What's bringing new butterfly species to Hong Kong? More questions than answers from *Euripes nyctelius*	Page 27

Tuesday, 12th June 2018

Pattern formation and Evo-Devo in Butterflies

Organizers	**Antonia Monterio**	**Abstract**
Plenary	**Fred Nijhout**	Page 15
Title	Wing Patterns: Discoveries and Puzzles	
09:00	Dasheri	

Venue		**Dasheri**	
Time	Speaker	**Talk Title**	Abstract
09:45	**Heidi Connahs**	*Distal-less* activates butterfly eyespots consistent with a reaction diffusion process	Page 28
10:00	**Oskar Brattström**	Mycalesina in morphospace: How developmental bias shape evolutionary diversification in butterflies	Page 28
10:15	**Vincent Debat**	Exploring the evolution of eyespots in *Morphos*	Page 29
10:30	**Karl Gotthard**	Local adaptation of plasticity for life cycle regulation in a temperate butterfly	Page 29
10:45	**Antónia Monteiro**	The origin of phenotypic plasticity in eyespot size in response to temperature	Page 30
11:00 – 11: 20		**Tea break**	
11:20	**Richard Gawne**	The Arctiid Archetype: A New Lepidopteran Groundplan	Page 31
11:35	**Michael Perry**	Expanding color vision: the developmental basis for the diversification of retinal mosaics in butterflies	Page 31
11:50	**Anupama Prakash**	*apterousA* is required for the differentiation of dorsal wing patterns in butterflies	Page 32
12:05	**Kenneth McKenna**	Lepidoptera wing shape evolves through changes in the relative growth of anterior and posterior compartments	Page 32
12:20	**Soumen Mallick**	Phenotypic variation in relation to climatic variation in an evergreen tropical rainforest butterfly	Page 33
14: 00	**Harshad Mayekar**	Decoupled reaction norms for two correlated pupal traits in a tropical satyrine butterfly	Page 33

Tuesday, 12th June 2018

Ecological Immunology in Butterflies

Organizers	**Anne Duplouy and Marjo Saastamoinen**
Venue	LH1 / Haapus

Venue		**LH1 / Haapus**	
Time	**Speaker**	**Talk Title**	**Abstract**
09:45	**Nora Braak**	How does a butterfly embryo cope with immune challenges?	Page 34
10:00	**Christopher Wheat**	Adaptive changes in immune genes in two butterflies, *Pieris napi* and *Pararge aegeria*	Page 34
10:15	**Wen-Hao Tan**	Signatures of Selection on Immunity-related Genes across Monarch Butterfly Populations	Page 35
10:30	**Juan Galarza**	Defensive symbiosis between bacteria and moths	Page 36
11:00 - 11: 20		**Tea break**	
11: 20	**Jacobus de Roode**	Effects of plant toxins on monarch butterfly infection, immunity and the gut microbiome	Page 36
11: 35	**Anne Duplouy**	Silk properties and overwinter survival in gregarious larvae of the Glanville fritillary butterfly	Page 37
11: 50	**Liam Murphy**	Immune-development trade-offs in the wood tiger moth *Arctia plantaginis*	Page 37
12: 05	**Elena Rosa**	A plant pathogen modulates the effects of secondary metabolites on the performance and immune function of an insect herbivore	Page 38

Tuesday, 12th June 2018

Colourful Stories: The How's and the Why's of Butterfly Structural Colours

Organizers	**Anupama Prakash and Seah Kwi**	**Abstract**
Plenary	**Bodo Wilts**	Page 15
Title	Photonic properties of brilliant butterfly displays: the role of pigments and disorder	
14: 15	Dasheri	

Venue		**Dasheri**	
Time	**Speaker**	**Talk Title**	**Abstract**
15: 00	**Nicola Nadeau**	*Heliconius* iridescence: natural hybrid zones elucidate the evolution and genetics of structural colour	Page 38
15: 15	**Yuji Matsuoka**	Melanin pathway genes regulate color and morphology of butterfly wing scales	Page 39
15: 30	**Rachel Thayer**	Genetic basis and evolutionary context for structural color shift in the Buckeye butterfly	Page 39
15: 45 – 16: 05		**Tea break**	

Open Symposium

Organizers	**Nathan Morehouse and Ramprasad Neethiraj**	**Abstract**
Venue	Dasheri	

Venue		**Dasheri**	
Time	**Speaker**	**Talk Title**	**Abstract**
16: 05	**Nathan Morehouse**	Coevolution of male and female reproductive proteins in the Cabbage White butterfly, *Pieris rapae*	Page 40
16: 20	**Camille Le Roy**	Evolution of morphologies and associated behaviours: how geometric morphometrics and kinematics can spread light on the evolution of *Morpho* butterflies wings	Page 40
16: 35	**Vaishali Bhaumik**	Female butterflies modulate investment in reproduction and flight in response to migration	Page 41
16: 50	**Ramprasad Neethiraj**	Allele specific expression reveals that the sex chromosome is overrepresented in a local adaptation phenotype	Page 42
17: 05	**Petr Nguyen**	Genome Instability in blue butterflies (Lycaenidae)	Page 42

Wednesday, 13th June 2018

Evolution and Diversification of Aposematism and Mimicry Systems

Organizers	Krushnamegh Kunte and Johanna Mappes	Abstract
Plenary	**Lawrence E. Gilbert**	Page 16
Title	Evolutionary innovations that define and diversify *Heliconius*	
09:00	Dasheri	

Venue		Dasheri	
Time	**Speaker**	**Talk Title**	**Abstract**
09: 45	**Thomas Sherratt**	The evolution of hidden colour signals in moths (and other insect groups)	Page 43
10: 00	**Violaine Llaurens**	Evolution of chemical defenses in the genus Heliconius	Page 43
10: 15	**Marianne Elias**	Ecological and evolutionary consequences of mimicry in Ithomiine butterflies	Page 44
10: 30	**Johanna Mappes**	Why do all extraordinary cases of mimicry come from the tropics? (or do they?)	Page 44
10: 45	**Krushnamegh Kunte**	Evolutionary assembly in butterfly mimicry rings	Page 45
11: 00 – 11: 20		**Tea Break**	
11: 20	**Anniina L.K. Mattila**	Trade-offs between chemical defense and life-history and fitness traits in a *Heliconius* butterfly	Page 45
11: 35	**Erica Westerman**	The genetics of assortative mating in *Heliconius cydno*	Page 46
11: 50	**Melanie McClure**	The role of pharmacology in unpalatbility, aposematism, and courtship of clearwing butterflies	Page 46
12: 05	**Charline Sophie Pinna**	The unexpected evolution of transparency in aposematic Lepidoptera	Page 47
12: 20	**Dipendra Nath Basu**	Evolution of flight morphology in butterfly mimicry rings	Page 47

Wednesday, 13th June 2018

Notes from the Field: Observations and Hypotheses

Organizers	**David Lohman**	**Abstract**
Plenary	**Yu-Feng Hsu**	Page 17
Title	The Agehana swallowtails: their biology, biogeography, and systematic	
14: 00	Dasheri	

Venue		**Dasheri**	
Time	**Speaker**	**Talk Title**	**Abstract**
14: 45	**Martin Wiemers**	Discovery of the life history of *Papilio demoleus sthenelinus* in the Lesser Sunda Islands	Page 48
15: 00	**Wayne Whaley**	Population biogeography of *Papilio indra* and its larval host plants	Page 48
15: 15	**Tsuyoshi Takeuchi**	Uncertainty about flying conspecifics causes territorial contests of the swallowtail *Papilio machaon*	Page 49
15: 30	**Michael Braby**	Some remarkable discoveries of butterflies and diurnal moths in the monsoon tropics of northern Australia	Page 49
15: 45	**Elena Pazhenkova**	Butterfly field observations resulted in evolutionary and taxonomic hypotheses	Page 50
16: 00 – 16: 20		**Tea Break**	
16: 20	**Alexander L. Monastyrskii**	Specific features of the butterfly fauna of Vietnam with emphasis on biogeography; field surveys 1994-2018	Page 50
16: 35	**Gerard Talavera**	Investigating the world wide migrations of the Painted Lady butterfly (*Vanessa cardui*) through integrative research	Page 51
16: 50	**Patricio Salazar**	*Tilapia* as a potential carrion bait for studies of tropical butterfly communities	Page 51
17: 05	**Robin Curtis**	The potential for lightweight unmanned aerial vehicles to revolutionize butterfly research	Page 52
17: 15		**Poster Session**	

Thursday 14th June 2018

Diversification, Speciation and Biogeography in the Tropics

Organizers	**Nicolas Chazot and Pavel Matos**	**Abstract**
Plenary	**Niklas Wahlberg**	
Title	20 years of molecular systematics: where are we with butterfly phylogeny now?	Page 17
09:00	Dasheri	

Venue		**Dasheri**	
Time	Speaker	Talk Title	Abstract
09: 45	**Niclas Backström**	Speciation and genome evolution in cryptic wood-white (Leptidea) butterflies.	Page 52
10: 00	**Keith Willmott**	From subtribal classification to cryptic species delimitation: progress in clarifying the systematics of the diverse *Euptychiina* butterflies (Nymphalidae, Satyrinae)	Page 53
10: 15	**Michael Braby**	Patterns of biodiversity of butterflies and diurnal moths in the monsoon tropics of northern Australia	Page 53
10: 30	**Mariana Pires Braga**	Host repertoire evolution and diversification of butterflies	Page 54
10: 45	**Pavel Matos**	Molecular systematics of *Haeterini* (Nymphalidae: Satyrinae): disagreements between the multispecies coalescent model and traditional taxonomic approaches	Page 55
11: 00 – 11: 20		**Tea Break**	
11: 20	**Erin Campbell**	Out of (species) bounds: genome-wide SNPs uncover phylogenetic inconsistencies in the genus *Speyeria*	Page 55
11: 35	**Nicolas Chazot**	Diversification of Nymphalidae butterflies: assembling the "big picture".	Page 56
11: 50	**Neil Rosser**	Modes of reproductive isolation depend on geography in a pair of *Heliconius* butterflies.	Page 56
12: 05	**Emmanuel Toussaint**	Skipper anchored phylogenomics: Progress toward a comprehensive evolutionary tree for an enigmatic family of butterflies	Page 57
12: 20	**Giovanny Fagua**	Host-plant and biogeographical patterns of *Choristoneura* Lederer (Lepidoptera: Tortricidae)	Page 58
12: 35 – 14: 00		**Lunch**	

Diversification, Speciation and Biogeography in the Tropics

Time	Speaker	Talk Title	Abstract
14: 00	**David Lohman**	ButterflyNet An integrative framework for comparative biology	Page 58
14: 15	**Venkat Talla**	Population genetics of cryptic European wood-white butterflies.	Page 59
14: 30	**Kwaku Aduse-Poku**	Evaluating the drivers of Afrotropical biodiversity; speciation and dispersal of a Satyrine butterfly group in a Paleoclimatic Pendulum	Page 60
14: 45	**Shuang Xing**	Treading the Wallacean shortfall for an endangered, iconic and in-demand tropical butterfly (*Teinopalpus aureus*)	Page 60
15: 00	**Samridhi Chaturvedi**	Does historical admixture predict patterns of introgression in a contemporary hybrid zone? A comparison of recent and ancient admixture in *Lycaeides* butterflies	Page 61
15: 15	**Vlad Eugen Dinca**	Using genome-wide representation and *Wolbachia* screening to understand high intraspecific levels of mitochondrial DNA divergence	Page 62
15: 30 - 15: 50		**Tea Break**	

Thursday 14th June 2018

Global Citizen Science Monitoring of Butterflies: Building a Globally Engaged Community for Science, Education and Conservation

Organizers	**Leslie Ries and Vijay Barve**	**Abstract**
Plenary	**David Roy**	Page 18
Title	What have we learnt from monitoring butterfly population in the UK?	
15: 50	Dasheri	

Venue		Dasheri	
Time	**Speaker**	**Talk Title**	**Abstract**
16: 35	**Leslie Ries**	The monarch butterfly as a model system for the use of citizen science monitoring data	Page 62
16: 50	**Vijay Barve**		Page 62
17: 05	**Doug Taron**	Global Protocols for Butterfly Monitoring	Page 63
17: 20	**Suman Attiwilli**	A citizen science butterfly monitoring programme in India: Temporal dynamics of butterfly assemblages in a changing urban landscape	Page 63
17: 35	**Sanjay Sondhi**	Butterflies and Moths of India…Inspiring Citizen Science	Page 64

Thursday 14th June 2018

Foraging Ecology of Butterflies

Organizer	**Anuj Jain**
Venue	LH1 / Haapus

Venue		**Dasheri**	
Time	**Speaker**	**Talk Title**	**Abstract**
09: 45	**Timothy Bonebrake**	Plant resources and disturbance effects on butterfly communities un urban parks	Page 64
10: 15	**Ana Salgado**	Host plant preference and performance across life stages in Glanville fritillarybutterfly (*Melitaea cinxia*)	Page 65
10: 30	**Po-An Lin**	Switching between resistance and tolerance: impact of drought stress on defense strategies of tomato	Page 65

Thursday 14th June 2018

Sensory Ecology of Butterflies and Moths

Organizers	**Emilie Dion**
11: 20	LH1 / Haapus

Venue		**Dasheri**	
Time	Speaker	**Talk Title**	**Abstract**
11: 20	**Emilie Dion**	Early exposure to new sex pheromone blend alters mate preference in female butterflies and in their offspring	Page 66
11: 35	**Laurence Després**	Hybrid speciation and adaptive radiation along an elevational gradient in alpine butterfly	Page 67
11: 50	**Yash Sondhi**	Hidden UV wing patterns and evolution of visual genes in diurnal and nocturnal Lepidoptera	Page 67

Poster Title	Participant	Affiliation
Developmental biology of *Idea malabarica* Moore on a woody climber *Parsonia alboflavecens*	Aishwarya Hebbale ash.kalasa@gmail.com	College of Horticulture, Mudigere, India
Brain size as an evolutionary driver of complex visual signals in butterflies	Amod Zambre zambr043@umn.edu	University of Miami, USA
The tale of two pairs: functional convergence for thermoregulation in *Polyommatus* butterflies	Anshuman Swain answain@terpmail.umd.edu	University of Maryland, College Park, USA
Patterns of butterfly diversity, richness and abundance in association with heterogenous habitats	Deepak Naik mr.deepakln@gmail.com	Mangalore University, India
Nuclear genes (but not mitochondrial DNA barcodes) reveal real species: evidence from the *Brenthis* fritillary butterflies (Lepidoptera, Nymphalidae)	Elena Pazhenkova epazhenkova@mail.ru	St. Petersburg State University, Russia
Demographic fluctuations lead to rapid and cyclic shifts in genetic structure among populations of an alpine butterfly, (*Parnassius smintheus*)	Jens Roland jroland@ualberta.ca	University of Alberta, Canada
Comparative genomics and lineage specific adaptations in Lepidoptera	Karin Nasvall karin.nasvall@ebc.uu.se	Uppsala University, Sweden
Development of biophotonic nanostructures in brush-footed butterfly wing scales	Kwi Shan Seah skwishan@hotmail.com	National University of Singapore, Singapore
Inferring the biogeography and demographic history of an endangered butterfly in Europe, *Coenonympha oedippus*, from multilocus markers	Laurence Despre´s laurence.despres@univ-grenoble-alpes.fr	University Grenoble Alpes, France
Inventory of butterfly fauna and distribution in Karbi Anglong District, Assam, India	Manashi Goswami mg3238@gmail.com	Assam University: Diphu Campus, India
Seasonal polyphenism in *Bicyclus dorothea* across different habitats in Cameroon	Michel Arnaud Kenfak Dongmo michelarnaud88@yahoo.fr	IITA/CBI, Cameroon
Evolution of larval host plant use by butterflies of the Western Ghats	Nitin Ravikanthachari nitin@ifoundbutterflies.org	NCBS, India
Evolution of wing colour at macroevolutionary scale in Mullerian mimicry butterflies	Ombeline Sculfort ombelinesculfort@hotmail.fr	UMR 7205, France
Effect of mating on survival of diapause females at low temperature in the Japanese common grass yellow	Tatsuro Konagaya konagaya@nibb.ac.jp	National Institute for Basic Biology, Japan
Differential expression of *engrailed* paralogs elucidates gene duplication events in butterflies	Tirtha Das Banerjee e0046855@u.nus.edu	National University of Singapore, Singapore
Convergence between the "metallic blue", *Euploea species* obscures the clarification of the model-mimic relationship	Shen-Horn Yen shenhornyen@mail.nsysu.edu.tw	National Sun Yat-sen University, Taiwan

Abstracts: Plenary talks

Symposium: Interacting Butterflies: From Genes to Communities
Organizers: **Robert Tropek** and **Marianne Espeland**

Plenary**: Naomi E. Pierce**, Harvard University, USA npierce@oeb.harvard.edu
Monday, 11th June 2018. 9:00 AM. Dasheri

Ecological specialization in the Lycaenidae and its role in diversification

In addition to specializing in host plant use like most butterflies, the caterpillars of many species in the family Lycaenidae have symbioses with ants that range from facultative to obligate, and from mutualistic to parasitic. These interactions are maintained by a number of complex behavioral adaptations. We use comparative phylogenetic methods and sequence variation to investigate the consequences of ant and/or host plant specialization at both a both a micro- and macroevolutionary scale. Ecological specialization appears to have promoted widespread diversification in the Lycaenidae, but under certain circumstances, may have also led to evolutionary dead-ends.

Symposium: Impact of weather trends and extremes on long-term population dynamics
Organizers: **Jens Roland** and **Steve Matter**

Plenary**: Marjo Saastamoinen**, University of Helsinki, Finland
marjo.saastamoinen@helsinki.fi
Monday, 11th June 2018. 14:00. Dasheri

Increased synchrony in weather conditions drives increased synchrony in metapopulation dynamics of the Glanville Fritillary butterfly in Finland

The long-term viability of metapopulations is dependent on independent dynamics of local populations, mitigating fluctuations in the metapopulation size as a whole. We studied if a recently reported increase in metapopulation synchrony of the Glanville fritillary butterfly in Finland could be explained by climate change induced change in synchrony of weather conditions. We used 23 years of survey data together with monthly weather records for the same period. We first examined the associations between population growth rates within different regions of the metapopulation and weather conditions during different life-history stages of the butterfly. We then examined the association between the spatiotemporal trends in the synchrony of these weather conditions and the synchrony of the metapopulation dynamics. I will first discuss how weather conditions impact the butterfly per capita growth rate. I will then show how the spatiotemporal increase in metapopulation synchrony is paralleled by an increase in the spatiotemporal synchrony of weather conditions, rather than caused by alternative explanations, such as increased dispersal or interactions with a specialist parasitoid. The climate driven increase in the Glanville fritillary metapopulation synchrony suggests that climate change can increase extinction risk of spatially structured populations living in fragmented landscapes by altering their dynamics.

Symposium: Pattern formation and Evo-Devo in Butterflies
(Special symposium in honour of Fred Nijhout)
Organizers: **Antónia Monteiro**

Plenary**: Fred Nijhout**, Duke University, USA hfn@duke.edu
Monday, 12th June 2018. 9:00 AM. Dasheri

Wing Patterns: Discoveries and Puzzles

Butterfly color patterns are built up from discrete pattern elements whose identity can be traced from species to species, and often across genera and families. Because of this identity it is possible to recognize homologies among pattern elements and to study their evolution and diversification. The shape, size and color of specific pattern elements can be under independent genetic control, and this has enabled the rapid and riotous diversification of wing patterns. I will review some of the salient aspects of pattern element evolution and outline several curious and still unresolved aspects of the development and evolution.

Symposium: Colourful Stories: The How's and Why's of Butterfly Structural Colours
Organizers: **Anupama Prakash** and **Seah Kwi Shan**

Plenary**: Bodo Wilts**, Adolphe Merkle Institute, Switzerland bodo.wilts@unifr.ch
Monday, 12th June 2018. 14:15. Dasheri

Photonic properties of brilliant butterfly displays: the role of pigments and disorder

The striking appearance of many butterflies is not obtained by pigments but rather by nanostructuring cuticle on the order of a few hundreds of nanometers. By changing the morphology of these nanostructures incident light can be manipulated in different ways giving rise to the brilliant displays of many butterflies. However, pigmentation is not negligible and can play an important role in further tuning and altering optical properties. Here, I will show the optical properties of different morphologies, from simple thin films to rather disordered structures and discuss the subtleties of pigmentation in a variety of butterflies.

Symposium: Evolution and Diversification of Aposematism and Mimicry Systems
Organizers: **Krushnamegh Kunte** and **Johannne Mappes**

Plenary**: Lawrence Gilbert**, University of Texas, Austin, USA lawrencegilbert0@gmail.com
Wednesday, 13th June 2018. 09:00. Dasheri

Evolutionary innovations that define and diversify *Heliconius*

One clade within the heliconiine lineage, passion vine butterflies, has undergone dramatic and recent diversification in the new world tropics. The life history innovation of pollen feeding appears to be a critical step in the emergence and radiation of genus *Heliconius*. This dietary innovation and subsequent metabolic rearrangements for larvae promoted adult palatability through enhanced cyanogenic defense. Rather than allocating plant-derived amino acids to egg production, *Heliconius* larvae allocate four amino acid precursors of CN glycosides to chemical defense of adults. One amino acid not deployed to cyanogen or silk protein, tryptophan, may increase its relative abundance in larval hemolymph. One way to reduce excess and harm of this potentially toxic amino acid is its conversion to the ommochrome pigments of brown, orange and red wing scales that uniquely characterize the wing pattern signals of the genus. Interactions of *Optix* and *Wnt* family regulatory genes provide a myriad of potential novel patterns highlighted by these xanthommatin-containing scales of *Heliconius*. Horizontal transfer of these regulatory genetic elements of a genus-wide toolbox between local races and species accelerates evolution of aposematic novelty and mimetic convergence.

Symposium: Notes from the Field: Observations and Hypotheses
Organizers: **David Lohman**

Plenary**: Yu-Feng Hsu**, National Taiwan Normal University, Taiwan t43018@ntnu.edu.tw
Wednesday, 13th June 2018. 14:00. Dasheri

The Agehana swallowtails: their biology, biogeography, and systematics

The Agehana swallowtails are a pair of Oriental swallowtail species frequently placed in their own genus, but now generally included in the large genus *Papilio*. There are two known species: *P. maraho* and *P. elwesi*. Due to their peculiar appearance and rarity, there has been considerable interest in many aspects of their biology, phenology, systematic status, origin, biogeography, and conservation. My students and I studied the Taiwan endemic *P. maraho* and determined that larvae of this rare species feed on Sassafras growing in the open. The species has no fixed generations involving irregular or prolonged diapause. We sequenced DNA barcodes of *P. maraho*, typical *P. elwesi*, and a population of *P. elwesi* (form cavaleriei) that resembles *P. maraho*. Haplotypes of the form cavaleriei are all shared with typical *P. elwesi*. By contrast, haplotypes of *P. maraho* are not shared with *P. elwesi*, but the genetic divergence of barcodes between these two taxa is small, ~0.4%. Using two mitochondrial and two nuclear genes, we reconstructed phylogeny of major groups of papilionid butterflies including both *P. marao* and *P. elwesi*, and calibrated divergence times among these groups. The Agehana group is nested within the *Pterourus* Group B of the New World, suggesting that they have an American origin. They split from their American ancestors about 18 Ma, coinciding during the early Miocene climatic optimum. This suggests that the ancestor of the Agehana group came to East Asia via dispersal via the Bering Land Bridge. The wing patterns of the Agehana swallowtails are distinct from all other members of the *Pterourus* Group B in America, and we hypothesize this results from mimicry of Aristolochia-feeding swallowtails that are sympatric or parapatric with the Agehana swallowtails.

Symposium: Diversification, Speciation and Biogeography in the Tropics
Organizers: **Nicolas Chazot** and **Pavel Matos**

Plenary**: Niklas Wahlberg**, Lund University, Sweden niklas.wahlberg@biol.lu.se
Thursday, 14th June 2018. 14:00. Dasheri

20 years of molecular systematics: where are we with butterfly phylogeny now?

20 years ago the field of molecular systematics was just beginning to take off. The first molecular studies for butterflies had been published and there were high expectations of resolving their phylogenetic relationships. I will go through the different stages of molecular systematic studies over the past two decades and synthesize how they have changed our understanding of butterfly phylogeny. One of the biggest surprises was the non monophyly

of Papilionoidea, with hespriids and hedylids coming within and Papilionidae being sister to the rest of the butterflies. The solidification of the family level classification has taken place with the one superfamily-seven family system (Papilionidae, Hedylidae, Hesperiidae, Pieridae, Lycaenidae, Riodinidae and Nymphalidae) being widely accepted in the biology community. Major papers on intrafamilial systematics have been published for all families except Lycaenidae, which is forthcoming. The stage is set for a full all-species phylogenetic hypothesis for butterflies.

Symposium: Global Citizen Science Monitoring of Butterflies: Building a Globally Engaged Community for Science, Education and Conservation
Organizers: **Leslie Ries** and **Vijay Barve**

Plenary**: David Roy**, Centre of Ecology and Hydrology, UK dbr@ceh.ac.uk
Thursday, 14th June 2018. 15:50. Dasheri

What have we learnt from monitoring butterfly population in the UK?

Assessing the changing state of UK butterflies is made possible through the skill and dedication of thousands of volunteer recorders. Their contributions to long-term, citizen-science recording and monitoring schemes ensure a sound basis to understand the causes of change in biodiversity. I will give an overview of two long-running schemes to assess the status of butterflies in the UK, both primarily run in partnership between the charity Butterfly Conservation and the research organisation, the Centre for Ecology & Hydrology. The UK Butterfly Monitoring Scheme celebrated its 40th anniversary in 2016, and the Butterflies for the New Millenium recording scheme comprises over 11 million butterfly occurrence records from 1690 to the present day. I will give a general overview of how the schemes have developed and their contribution to understanding the impacts of environmental change on butterflies, as indicators of wider biodiversity. I will describe key research papers that provide some of the best evidence of the impacts of climate change, including extreme events, on biodiversity. I will conclude with my personal view on the priorities for future development of citizen science butterfly monitoring schemes, in the UK and with potential relevance to other parts of the world.

Abstracts: Symposia talks

Symposium: Interacting Butterflies: From Genes to Communities
Organizers: **Robert Tropek** and **Marianne Espeland**

Tempo and mode of butterfly host-driven diversification

Niklas Janz

Department of Zoology, Stockholm University, Sweden niklas.janz@zoologi.su.se

Butterflies are among the best known examples of host-driven diversification, yet there has also been some controversy around the tempo and mode of this diversification. The proposed hypotheses can broadly be categorized into two main alternative scenarios. The first, the "explosive" radiation scenario☐, is dominated by episodic bursts of diversification (radiations) that typically follow host shifts. In the other, the "plasticity scenario"☐, diversification is more constant and less dramatic, and is driven by plasticity in host use. The main differences between the scenarios can be boiled down to 1) the extent to which novel hosts represent new adaptive zones that support radiation, and 2) the extent to which ancestral hosts can remain as latent abilities to support future recolonization when ecological opportunities change. I will try to outline these main differences, and suggest how they can both be unified into a common general framework.

Understanding persistence on a novel host in the context of phytochemistry, microbes and arthropod communities

*M. L. Forister**, *C. A. Buerkle, C. D. Dodson, S. Chaturvedi, J. A. Fordyce, Z. Gompert, S. Lebeis, L. Lucas, Z. Marion, K. Moccia, C. C. Nice and C. Philbin*

*Department of Biology, University of Nevada, USA forister@gmail.com

Host range dynamics are central to issues that include diversification, specialization and the persistence of populations in the Anthropocene. Outstanding questions in this area include the relative importance of different host traits in the colonization process, as well as the underlying genetic architecture associated with the use of alternative host plants. Here we present a detailed look at a single instance of host range expansion: the colonization of exotic alfalfa by the Melissa blue butterfly, *Lycaeides melissa*. Axes of plant variation explored include detailed phytochemistry, genetic diversity, structural and phenological traits, as well as associated fungal and bacterial communities. *L. melissa* persists on the novel host in a delicate balance of inter-specific interactions, while genome evolution proceeds and potentially restricts future host associations. Results are discussed in the context of classic and recent hypotheses on the evolution of ecological specialization.

Genetic basis of a female-limited alternative life history switch & insights into the biotic interactions maintaining it within populations

Alyssa Woronik, Kalle Tunstram, Ramprasad Neethiraj, Michael W. Perry, Constanti Stefanescu, Maria de la Paz Celorio-Mancera, Jason Hill, Oskar Brattström, Philipp Lehmann, Reijo Käkelä and Christopher W. Wheat[*]

[*]Stockholm University, Sweden chris.wheat@zoologi.su.se

Alternative life history strategies (ALHS) are found across diverse species, where one sex exhibits discrete differences in phenotype due to divergent investments in growth and reproduction, that lead to trade-offs directly impacting Darwinian fitness. ALHS are well documented in males and have long attracted the attention of ecologists and theoreticians. In contrast, few examples of female-limited ALHS are known, either due to biological reality or our lack of understanding of how ALHS manifest in females, highlighting the need for mechanistic insights, as none exist for female ALHS. Here we identify the genetic basis a female-limited ALHS. A single genomic region in the butterfly *Colias croceus* controls the switch between ALHS morphs where females trade-off resources between reproduction and orange pigment synthesis, producing wing color phenotypes (orange vs. white, known as Alba). We find Alba is a gain of function mutation due to a transposable element insertion downstream of the homeobox transcription factor, BarH-1, whose knockout in *D. melanogaster* changes eye color from WT red to white by affecting pigment granule development. Here, CRISPR/Cas9 knockout of BarH-1 in *C.croceus* confirms a role in eye pigmentation and reveals a novel function in wing morphology, as the orange/white switch arises via a reduction in pigment granules within Alba wing scales. In order to gain insights into the mechanisms maintaining this variation within populations, we have begun to generate Alba specific markers for unbiased estimates of Alba frequencies within populations over time. Additionally, we present results from longterm (> 20 years) field data of Alba abundance over time, along with sympatric butterfly species, which suggest an important role of the butterfly community as a whole in maintaining this female-limited alternative life history switch.

Role of butterflies in pollination networks of Mount Cameroon

Robert Tropek[*]*, Jan Mertens , Yannick Klomberg , Jan Raska and Stepan Janecek*

[*]Faculty of Science, Charles University, Czech Republic robert.tropek@gmail.com

Butterflies play a crucial role in many pollination systems, although they are not among the most dominant pollinators of most plants. Especially some groups of butterflies are common visitors of flowers, with consequences for their ecology and evolution. However, the role of butterflies in pollination systems within entire communities is only rarely quantified, especially under different environmental conditions. In the past two years, we have been recording pollination interactions along a complete rainforest altitudinal gradient of Mount

Cameroon. During both dry and wet seasons, we systematically record visitors of all flowering plant species in communities in different altitudes from lowland to timberline, resulting in already 1,200 24-h videorecordings of over 200 plant species. Our contribution will focus on butterflies (>200 records of 20 butterfly species on 20 plant species) in montane and submontane forests. We will quantify the importance of butterflies in pollination systems in different altitudes and seasons. Furthermore, we will analyse changes in specialisation of individual butterfly and plant species, with consideration of their morphological, behavioural and other functional traits. Finally, we will test the validity of psychophilous pollination syndrome under different environmental conditions, with a potential interpretation regarding the evolution of butterfly-plant pollination interactions.

Host plant abundance explains negative association between larval performance and female preference

*Ranjit Kumar Sahoo*and Tenzing Doleck*

*School of Biology, IISER Thiruvananthapuram, India sahoork@iisertvm.ac.in

Widely distributed polyphagous insects provide opportunities to understand the influence of the local plant communities on the geographic variation in the insects host plant use pattern. Larval performance in the widespread butterfly *Acraea terpsicore* appears to vary geographically. This prompted us to investigate in detail host plant associated divergences in behaviour and life history traits between two Indian populations of this species. Because the two populations differ in the abundances of the host plants, we tested whether local availability of host plants has influenced evolution of larval performance and female oviposition preference. We also asked whether females prefer to oviposit on the host plants that are optimal for larval development. We find that host plants influence larval performance similarly for both the populations. Female preference does not correlate with larval performance; however, the preference varies among the populations. In one population, the females preferred the locally abundant host plant for oviposition although the plant was sub-optimal for larval development. This negative preference-performance correlation indicates that high searching cost for the optimal host plant has probably influenced female behaviour.

Butterfly male killers: Intracellular bacterium induces female biased sex ratio in a butterfly

Kruttika Phalnikar

National Centre for Biological Sciences, India kruttikap@ncbs.res.in

In insects, sex ratio can get altered in a very unique and interesting manner, which is via bacterial infection. Many insects are infected with *Wolbachia*, a genus of obligate intracellular bacteria that are transferred to subsequent generations via insect eggs. *Wolbachia* can drastically alter the sex ratio of insects via reproductive manipulations like male-killing and feminization that lead to female biased populations. This way, *Wolbachia* achieves higher rate of transmission to the next generation, thus increasing its own fitness. Here we report a new case of reproductive manipulation by *Wolbachia* in butterfly *Ariadne merione*. We found that *A. merione* shows a strong female bias and is heavily infected with *Wolbachia*. We tested whether *Wolbachia* induces female bias in *A. merione* by treating butterfly larvae with antibiotics. We found that elimination of *Wolbachia* not only reverses the female bias but results in a strong male bias. Such a drastic sex ratio reversal might stem from a phenomenon where females cannot survive without *Wolbachia* whereas males cannot survive with *Wolbachia*. Overall, we predict that interaction between *Wolbachia* and *A. merione* will unravel a new mechanism of reproductive manipulation and can have a substantial impact on *A. merione* biology.

A geographic mosaic of *Wolbachia* infection in *Melanitis leda* butterfly populations

*David J. Lohman*and Brandon LaTorre*

*City College of New York, USA dlohman@ccny.cuny.edu

Insects are the most species-rich multicellular taxon on the planet, and it is estimated that more than half of all insect species are infected with the endosymbiont *Wolbachia*. This bacterium can manipulate the reproduction of its host and potentially affect host evolution. Most surveys investigating patterns of *Wolbachia* infection in nature sample limited numbers of individuals; population-level investigations into geographic differences in infection status that might affect host evolution are few. To investigate geographic variability among populations of a single species, we assayed 133 *Melanitis leda* butterflies (Lepidoptera: Nymphalidae: Satyrinae) for *Wolbachia* infection collected throughout its range in the Old World tropics and subtropics from Ghana to Fiji. Potential effects of the parasite on the evolution of its host were assessed by inferring phylogenies of the host with nuclear and mitochondrial markers, and by inferring relationships among the *Wolbachia* that we detected in different populations. Geographic variability was apparent on two levels: prevalence of infection and sequence type (strain, ST) of infection. All *M. leda* on Java and all landmasses south and east of Wallace's Line were infected with at least one of multiple sequence types; individuals in Australia and Fiji hosted at least two different sequence types per individual. Butterflies collected elsewhere were sometimes infected with a different sequence type, but were often not infected. Infection status coincided with patterns of nuclear and mitochondrial variability. There was no phylogenetic structure in populations collected throughout Africa, Madagascar, South Asia, mainland Asia, the Philippines, Borneo, and Sumatra, implying frequent gene flow. Specimens from Australia, New Guinea, and the archipelagos of Southeast Asia and Oceania had nuclear and mitochondrial phylogenetic structure. The topology of nuclear and mitochondrial trees conflicted with regard to the placement of the clade containing specimens from the South Pacific and Ghana, implying selection imposed by the endosymbiont in these regions. These results suggest that marine barriers limit the dispersal of the host, which permits different strains of *Wolbachia* to enter semi-isolated populations through horizontal transmission at different times and places before subsequent vertical transmission and differential effects on its hosts' evolution.

The missing association between butterflies and their gut bacteria

Kruttika Phalnikar, Krushnamegh Kunte and Deepa Agashe[*]

[*]National Centre for Biological Sciences, India dagashe@ncbs.res.in

Insects occupy diverse dietary niches, and a large body of work suggests that this ability to use different diets depends on strong associations with specific gut bacteria. Butterflies present an interesting case where dietary shifts occur not only across species, but also across development (most larvae are phytophagous and adults consume liquids). Hence, we predicted that bacterial communities of butterflies should show strong host species-specific as well as stage-specific signatures. As expected, across12 wild-caught butterfly species, we found that host taxonomy explained a significant amount of variation in bacterial community structure. For adult butterflies, dietary guild (e.g. nectar-feeding vs. non-nectar feeding) also explained bacterial communities. Surprisingly, across 9 species where we could sample multiple life stages, we found that caterpillars and adults had very similar bacterial communities; and the communities of caterpillars strongly resembled those of their host plant. These results suggest largely passive, diet-driven community assembly across butterfly life stages, with little role for subsequent host filtering. This hypothesis is supported by results from manipulative experiments: for two butterfly species, disrupting the bacterial communities of caterpillars had negligible fitness impact. Together with recent work on neotropical butterflies, our analyses highlight butterflies as an interesting insect clade that evolved to use a spectacular diversity of dietary resources without establishing significant relationships with microbes.

The impact of mutualistic and parasitic life histories on butterfly diversification in an increasingly arid world

Marianne Espeland[*]*and Naomi Pierce*

[*]Zoological Research Museum Alexander Koenig. Germany m.espeland@leibniz-zfmk.de

75% of all lycaenid butterflies are associated with ants. These interactions are mainly mutualistic: Ants receive nutritious secretions from glands on the cuticle of the caterpillar, and in return protect the caterpillars against predators and parasites. Mutualistic interactions have evolved into parasitism multiple times independently, where the caterpillars eat the ant brood or are being fed mouth-to mouth by the ants. The evolution of parasitism occurs mainly in single species in otherwise mutualistic clades, and parasitism has therefore been seen as an evolutionary dead-end. The Afrotropical Euchrysops section with around 210 species in all habitats from rainforest to semi-deserts includes both parasitic and

mutualistic taxa. They started diversifying around 14 ma in the mid Miocene at the beginning of the aridification of Africa, and subsequently dispersed into dryer habitats as these became available. Most extant taxa evolved during the last 5 Myr, and there was a major increase in diversification rate at the base of the large parasitic clade (138 spp. in arid areas) around this time, indicating that the evolution of parasitism might be a key innovation for life in arid environments, by providing a stable food source during times of the year when very little vegetation is available.

Symposium: Impact of weather trends and extremes on long-term population dynamics
Organizers: **Jens Roland** and **Steve Matter**

Butterflies and climate: regional declines, local responses, and increasing unpredictability

James A. Fordyce, Matthew L. Forister, Christopher C. Nice, Zachariah Gompert, Joshua G. Harrison, James H. Thorne, David P. Waetjen and A. M. Shapiro*

*University of Tennessee, USA jfordyce@utk.edu

Some responses to climate change have been well characterized, such as shifts in phenology and geographic distribution. However, variation in population-level responses to climatic variation are less well understood. We examined heterogeneity in response to climate variation using a long-term dataset (42 years) that includes 149 butterfly species across 10 sites encompassing considerable habitat heterogeneity, ranging from sea level to over 2700 meters in California. We found extreme heterogeneity in butterfly population responses to climatic variation across sites and among species within sites. Despite the high observed heterogeneity in response to climate, our model had strong predictive ability, though this was compromised during a period of extreme drought. Our results suggest that habitat heterogeneity may buffer some of the negative consequences of climate change. These results highlight the importance of long-term monitoring to inform models of population dynamics that are the foundation of biodiversity management in a rapidly changing world.

The effects of temperature and precipitation on population dynamics of both common and endangered butterflies

Erica Henry, Heather Cayton, Elsita Kiekebusch, Tyson Wepprich and Nick Haddad*

*North Carolina State University, USA ehenry@ncsu.edu

Warmer temperatures and changes in precipitation have the potential to alter the number of generations a butterfly population completes in a year. It has been hypothesized that an additional generation can rapidly lead to population extinction if these individuals do not have sufficient time to reach diapause stage and experience high mortality. We test this lost generation hypothesis with three groups: 1) common species whose phenologies are controlled by temperature (20 species from Ohio, USA); 2) a rare species whose phenology is controlled

by temperature (St. Francis' satyr); and 3) a rare species whose phenology is controlled by rainfall (Miami blue). Instead of decreasing population growth rates, we found increased voltinism resulted in increased population growth rates for 13 of the 20 common species, with only one species exhibiting a decline in population growth rate. This pattern holds true for St. Francis' satyrs as well. For Miami blues, we discovered that uncertainty in our knowledge of the butterfly's natural history masks any potential effects changes in climate-driven phenology patterns have on population persistence. Results of these studies suggest that increasing voltinism in response to warming temperatures may help species adapt to climate change, at least for temperature driven species.

Early-winter extreme weather dominates dynamics of alpine *Parnassius smintheus* butterflies in the Rocky Mountains of Canada

Jens Roland[*]*and Steve Matter*

[*]University of Alberta, Canada jroland@ualberta.ca

We use long-term (23 years) data from 21 sub-populations of the alpine butterfly, *Parnassius smintheus*, to identify climate and weather variables that relate most strongly to annual rates of change (Rt) between summer adult butterfly populations. Using a combination of machine learning (RandomForests) and mixed-effects models, we found that extreme temperatures interacting with snow fall amount in November affect annual change most strongly. Populations decline in years with extreme cold and hot events in November, particularly in years with little regional snow cover. Results indicate that over-wintering eggs are particularly vulnerable to extreme temperatures as they enter winter. Using high-resolution satellite imagery, we confirm that spatial variation in snow cover among meadows (and within years) in late November dominates annual population change. Increased variation in early-winter extreme temperatures (both hot and cold), coupled with a pattern of reduced snow pack, place these alpine populations as risk. By definition, the effects of rare events on populations are detectable only with sufficiently long time-series.

Let it snow, let it snow, but not too much: Overwintering survival affects population growth of *Parnassius smintheus*

Stephen F. Matter[*]*, Kelsey Harrison and Jens Roland*

[*]University of Cincinnati, USA mattersf@uc.edu

Past research has shown that the population growth of the butterfly *P. smintheus* is related to both climatic conditions and extreme weather events during its overwintering life stage. It has been assumed that the temperature on the ground, where butterflies overwinter, affects overwintering survival, but direct assessment has been lacking. Here we demonstrate how warmer overwintering temperatures decrease overwintering survival and population growth.

El-niño, host plant growth and migratory butterfly abundance

Robert B. Srygley,[*] *Robert Dudley, Evandro G. Oliveira and Andre J. Riveros*

[*]USDA-Agricultural Research Service, USA robert.srygley@ars.usda.gov

In the wet forests of Panama, El-niño typically brings a more prolonged and severe dry season. Interestingly, many trees and lianas that comprise the wet forests increase their productivity as a response to El-niño. Here we quantify the abundance of migrating *Marpesia chiron* butterflies over 17 years and the production of new leaves of their hostplants over 9 years to test the generality of the El-niño migration syndrome, i.e., whether increased abundance of migrating insects and productivity of their food plants are associated with El-niño and La Niña events. We find that the quantity of *M. chiron* migrating across the Panama Canal was directly proportional to the sea surface temperature (SST) anomaly of the Pacific Ocean, which characterizes El-niño and La Niña events. We also find that production of new leaves by its larval host trees, namely *Brosimum alicastrum*, *Artocarpus altilis*, and *Ficus citrifolia*, was directly proportional to the SST anomaly, with greater leaf flushing occurring during the period of the annual butterfly migration that followed an El-niño event. Combining these and our previously published results for the migratory butterfly *Aphrissa statira* and its host lianas, we conclude that dry season rainfall and photosynthetically active radiation can serve as primary drivers of larval food production and insect population outbreaks in Neotropical wet forests, with drier years resulting in enhanced plant productivity and herbivore abundance. Insect populations should closely track changes in both frequency and amplitude of the El-niño Southern Oscillation with climate change.

Conservation genetics of the Marsh Fritillary butterfly *Euphydryas aurinia* in Great Britain

Michelle L Davis,[*] *Ian Powell, Keith Porter and Paul A Ashton*

[*]Edge Hill University, UK davism@edgehill.ac.uk

Species with short generational times such as Lepidoptera are at risk from rapid loss of genetic diversity when population sizes decline. Although conservation efforts can lead to recovery of the population size this does not recover the lost genetic diversity and leaves the species at a greater risk from changes to its environment due to inability to respond to selection. *Euphydryas aurinia* (Marsh Fritillary) was once widespread in the UK but has declined over the past hundred years, with particularly severe declines noted form the 1980s onwards. In the UK *E. aurinia* is now highly fragmented and restricted primarily to western Scotland and south-western England, with a few other isolated populations. This distribution presents the opportunity to examine the effect that changes in population size have on the genetic diversity. Some populations have remained stable for decades while others have declined and there has been a successful reintroduction in northern England. We will compare the genetic diversity at three sites which cover the range of population scenarios; stable, declining and reintroduced. We will also consider evidence for gene flow

between Great Britain and Ireland and, via museums specimens, the historic levels of genetic diversity within British *E. aurinia* populations.

Misinformation in a new climate: Photoperiod-induced seasonal polyphenism under climate change

*Matthew Nielsen**and Joel Kingsolver*

*University of North Carolina, USA maerniel@live.unc.edu

Phenotypic plasticity requires reliable cues that predict the future environment to produce adaptive phenotypes, but these cues may be indirect. Many temperate species, including butterflies, use photoperiod as an indirect cue for seasonal plasticity. Climate change, however, has increased temperatures without changing photoperiod itself, reducing the effectiveness of photoperiod as a cue. Evolution could compensate for this cue-environment mismatch by altering the plasticity reaction norm, but has this happened? I am addressing this question in *Colias eurytheme* butterflies, a species with thermoregulatory seasonal polyphenism, but which uses photoperiod, not temperature, as a cue. I am using photographs of specimens from natural history collections to look for variation *C. eurytheme* wing pattern at different times of year over the last seven decades of climate change. Any changes found, particularly in the time of year at which the different phenotypes are found, would have to reflect some degree of evolutionary change in the reaction norm since the original cue, photoperiod, has not changed. I am also directly testing for a change in how *C. eurytheme* responds to photoperiod and temperature by recreating the original 1970s experiments which measured C. *eurytheme's* response to these factors but using contemporary populations.

What's bringing new butterfly species to Hong Kong? More questions than answers from *Euripes nyctelius*

Timothy Bonebrake

The University of Hong Kong, Hong Kong tbone@hku.hk

A common response to a warming climate in butterflies is through distribution shifts. Hong Kong has documented records of new butterfly species in recent decades, comprising tropical species and some of which have become successfully established. In this study, we examined the possible drivers for the establishment of *Euripus nyctelius* by studying its thermal physiology and modeling current climate and future distributions projected by species distribution modelling (SDMs). We corroborated that *Euripus nyctelius* has a significantly higher critical thermal minimum than temperate relatives (in this case *Hestina assimilis*), suggesting a possible physiological constraint that may have been lifted with recent climatic warming. SDMs, however, did not provide strong evidence that a shifting climate envelope may be changing the suitability of climate for *Euripus nyctelius*. We hypothesize that habitat changes in the region, potentially acting in concert with climate change, have led to the expansion of *Euripus nyctelius*. In sum, what led to the establishment of this species and others in recent years in Hong Kong remains a mystery. Tropical butterfly assemblages require a significant advancement in efforts to monitor species and populations if we are to conclusively document climate-driven shifts in species distributions.

Symposium: Pattern formation and Evo-Devo in Butterflies
(Special symposium in honour of Fred Nijhout)

Organizers: **Antónia Monteiro**

Distal-less activates butterfly eyespots consistent with a reaction diffusion process

Heidi Connahs, Sham Tlili, Jelle van Creij, Tricia Y. J. Loo, Tirtha Banerjee, Timothy E. Saunders and Antónia Monteiro*

*National University of Singapore, Singapore hconnahs@gmail.com

Eyespots on the wings of nymphalid butterflies represent colorful examples of pattern formation, yet the developmental origins and mechanisms underlying eyespot differentiation are still poorly understood. Using CRISPR-Cas9 we re-examine the function of Distal-less (Dll) as an activator or repressor of eyespots, a topic that remains controversial. We show that the phenotypic outcome of CRISPR mutations depends upon which specific exon is targeted. In *Bicyclus anynana*, exon 2 mutations are associated with both missing and ectopic eyespots and also exon-skipping. Exon 3 mutations, which do not lead to exon-skipping, produce only null phenotypes including missing eyespots, lighter wing coloration and loss of scales. Furthermore, reaction-diffusion modeling of Dll's function accurately replicated mutant phenotypes, identifying this molecule as the first transcription factor involved in a reaction-diffusion process leading to eyespot formation. These results clarify the function of Dll as a required activator of eyespot development, scale growth and melanization, and reveal how the tuning of Dll expression levels can generate a diversity of eyespot phenotypes, including their appearance on the wing.

Mycalesina in morphospace: How developmental bias shape evolutionary diversification in butterflies

Oskar Brattström, Kwaku Aduse-Poku, Erik van Bergen, Vernon French and Paul M Brakefield*

*University of Cambridge, UK ob269@cam.ac.uk

We study how developmental bias can shape diversification in several related genera of butterfly. Experimental evolution using *Bicyclus anynana* revealed the potential for developmental bias in the evolution of repeated elements of wing eyespots; each eyespot forms around a central "organizer". Whereas the relative size of eyespots behaves in a highly flexible and unbiased manner, the colour composition of different eyespots shows strongly biased responses to selection in morphospace. We have now analysed evolutionary diversification in eyespot patterns over the whole subtribe of Mycalesina which comprises

over 300 species, including *B. anynana*. We have focussed on the size of eyespots and the relative size of individual colour elements (eyespot colour composition). Whilst most differences in patterning are not very substantial, species of the genus *Heteropsis* on Madagascar frequently show a highly enlarged yellow ring element in a single eyespot, such that this genus occupies a unique area of morphospace. Transplantation experiments in pupae reveal that this novel phenotype develops through modified responses in the wing epidermis to the inductive signals from the organizer. This reveals that potential developmental bias through shared development can contribute to shaping diversification, but on a macro-evolutionary scale such bias can be broken through novel innovations.

Exploring the evolution of eyespots in *Morphos*

Vincent Debat[*]*, SilvÃ¨re Jarosson, Patrick Blandin and Violaine Llaurens*

[*]Muséum national d'Histoire naturelle, France debat@mnhn.fr

Eyespots are well-documented butterfly color pattern, from developmental genetics to ecological significance. Ventral eyespots are generally viewed as deflectors of predator's attacks away from the body. Nevertheless, the diversity of eyespots within and across species questions the evolutionary forces at play. Here we investigate the evolution of eyespots in the neotropical genus *Morpho*. *Morphos* display series of ventral eyespots, highly variable among species, ranging from large, conspicuous structures to relictual spots barely qualifying as eyespots. Applying geometric morphometrics to a large sample (NÂ =Â 1038) spanning all *Morpho* species and both sexes, we assess eyespots number and position, and covariation with wing shape. We then focus on color, size and shape of the Rs eyespot shared by all species. We test whether color pattern covaries with wing shape and whether differential selection on eyespots occurs across the phylogeny. We detect a significant influence of wing shape on color pattern. Nevertheless, we find a significant increase in variation in eyespot number, position, size and shape in species with the smallest eyespots, suggesting relaxed selection on small eyespots. We hypothesize that contrasted ecology may explain the observed difference between species with large and phenotypically stable eyespots, acting as deflectors and species with small eyespots, faded in the pattern background or forming a thin line on pale species. We discuss these findings in the light of the (limited) knowledge on *Morphos'* ecology.

Local adaptation of plasticity for life cycle regulation in a temperate butterfly

Olle Lindestad, Christopher W. Wheat, Sören Nylin and Karl Gotthard[*]

[*]Dep. of Zoology, Stockholm University, Sweden karl.gotthard@zoologi.su.se

Seasonality varies in space and insects must express locally optimal life cycles, which typically means producing an annual number of generations (voltinism) appropriate to the local season length. Variation in voltinism may arise from direct effects of differences in temperature, or may result from local adaptation in reaction norms for life cycle regulation. Here we demonstrate substantial genetic differentiation in plasticity for life cycle regulation among populations of the Speckled wood butterfly, *Pararge aegeria*, across voltinism shifts at a relatively small spatial scale. These differences in plasticity are present both for the regulation of larval development rate and diapause induction. Using this data we parameterized a mechanistic phenological model for a set of populations within a single degree of latitude and show that even if passive and cue-like effects of temperature were present, the key determinant of local voltinism was heritable interpopulation differences in photoperiodic plasticity. These results demonstrate that local adaptation of reaction norms for life cycle regulation may evolve also across short geographic distances, as a result of selection for differences in voltinism. Moreover, they highlight the importance of incorporating local adaptation in life cycle regulation when trying to model and predict variation in phenological patterns in general.

The origin of phenotypic plasticity in eyespot size in response to temperature

Shivam Bhardwaj and Antónia Monteiro[*]

[*]National University of Singapore, Singapore antonia.monteiro@nus.edu.sg

There are two disparate views regarding phenotypic plasticity. One regards plasticity as a derived adaptation to help organisms survive in variable environments, while the other views plasticity as the outcome of flexible, non-canalized, developmental processes ancestrally present in most organisms, helping them colonize or adapt to novel environments. Both views of plasticity currently lack a rigorous, mechanistic examination across a phylogeny to understand ancestral and derived states and direction of change. Here I will show that the origin of phenotypic plasticity in eyespot size in response to environmental temperature observed in *Bicyclus anynana* butterflies is a derived adaptation of this lineage of butterflies. *B. anynana* displays plasticity in their eyespots in response to seasonal variation in the direction of both natural and sexual selection. The plasticity is mediated by a steroid hormone whose titers vary with rearing temperature. By examining the origin of each of the known physiological and molecular components of eyespot size plasticity in a comparative framework across 12 nymphalids and an outgroup, we observe that hormonal titer plasticity in response to temperature is a pre-adaptation shared by all butterfly species examined, whereas the origin of expression of the hormone receptor in eyespot centers, and the origin of eyespot sensitivity to the hormone-receptor complex, all essential components of the plastic response, are both derived traits found only in a subset of species with eyespots. The presence of all three molecular components required to produce a plastic response is only observed in the representative satyrid *B. anynana*. This gradual, step-wise, physiological/molecular response to temperature is a likely adaptation to natural temperature variation experienced across wet and dry seasons in the habitat of this species. This work supports, thus, the first view of plasticity as a derived adaptation.

The Arctiid Archetype: A New Lepidopteran Groundplan

Richard Gawne[*]*and H. Frederik Nijhout*

[*]Konrad Lorenz Institute for Evolution and Cognition Research, Austria richard.gawne@kli.ac.at

The nymphalid groundplan has proven to be an extraordinarily useful tool for the study of butterfly color patterns, and although it is widely assumed to be applicable to the moths, this conjecture is largely untested. Focusing on the hyper-diverse arctiid moths, we show that the patterns of this subfamily can be parsed into a limited number of apparently homologous characters. Some of these elements are positioned in a manner that corresponds to the arrangement of the nymphalid characters, but their morphology is often distinct. Symmetry systems are only found in a handful of arctiid genera, and other nymphalid characters such as the border ocelli appear to be absent altogether. To further research on moth wing patterns, and introduce the arctiids as a study system for evolutionary developmental biology, we develop a new theoretical groundplan known as the arctiid archetype that provides a framework for understanding wing patterning within this subfamily. Based on experimental studies of the 'bella moth' *Utetheisa ornatrix*, we hypothesize that wnt signaling could play an important role in the generation of arctiid wing patterns, and show how difference in wing shape and vein configuration can contribute to pattern diversity.

Expanding color vision: the developmental basis for the diversification of retinal mosaics in butterflies

Michael W. Perry[*]*, Cara Genduso and Claude Desplan*

[*]New York University, USA mp185@nyu.edu

Butterfly retinas are more complex than those found in flies such as Drosophila, allowing them to make additional color comparisons. We previously showed that the recruitment of an additional R7 photoreceptor cell during development allows butterflies to produce three stochastically distributed types of ommatidia (unit eyes) instead of the two found in Drosophila. We examine the developmental basis of this modification, and provide evidence that butterflies retain a transient cell known as the "mystery cell" in Drosophila as an additional R7-type photoreceptor. In butterflies, neighboring cells have modified expression of the transcription factors Spalt, Rough, Lozenge, and Bar. We test the genetic relationships between these factors in *Drosophila* and use CRISPR/Cas9 knockout in the butterfly *Vanessa cardui* to uncover their role in R7 recruitment. Sevenless plays a conserved role in R7 recruitment, but unexpectedly, sevenless knockout causes the loss of additional photoreceptors, suggesting an increased reliance on Sevenless signaling. We find that Lozenge expression is essential for recruitment of both R7s, and ongoing experiments will determine whether any single change in expression of candidate factors such as Lozenge are sufficient to turn on the developmental program to recruit a second R7 in place of the "mystery cell" in Drosophila.

***apterousA* is required for the differentiation of dorsal wing patterns in butterflies**

Anupama Prakash

National University of Singapore, Singapore anupama@u.nus.edu

Butterflies are colourful insects that show extensive variation in patterns between the dorsal and ventral wing surfaces. However, the molecular and developmental basis of this surface-specific patterning has not been investigated. A candidate gene for surface-specific wing patterning is *apterous*, a gene exclusively expressed on the dorsal wing surface of Drosophila melanogaster, a species with transparent wings. To test the functional role of apterous in butterflies and its potential involvement in wing patterning, we used the CRISPR-Cas9 system to disrupt two copies of *apterous*, *apterousA* (apA) and *apterousB* (apB), found in the genome of the African squinting bush brown, *Bicyclus anynana*. apA mutant individuals developed ventral wing patterns on the dorsal surface and displayed improper development of male-specific pheromone dispersing scales, suggesting a role for apA in specifying dorsal identities by being a repressor of ventral wing patterns as well as an activator of dorsal sex-specific traits. We propose that the co-option of apA into wing patterning and pigmentation pathways may have led to the extensive surface-specific modifications we see in butterfly wing patterns today. In addition, its role as a repressor of eyespots may have affected eyespot number diversity and evolution within the Nymphalids, by creating developmental constraints.

Lepidoptera wing shape evolves through changes in the relative growth of anterior and posterior compartments

*Kenneth Z. McKenna*and H. Frederik Nijhout*

*Duke University, USA kzm@duke.edu

Change in size and shape represents one of the most common evolutionary trends. In Butterflies and moths, a great diversity in wing morphology exists. Evolutionary changes in wing shape likely occur through changes in the spatial and temporal patterning of cell proliferation on the wing. Such patterning is due to the differentiation of anterior and posterior compartments. In Drosophila, A-P patterning requires the reciprocal interactions between the secreted morphogens wingless (wg), hedgehog (hh) and decapentaplegic (dpp). In Lepidoptera, wg is secreted from cells at the dorsal-ventral boundary, initiating the release of hh. Hh is secreted from cells in the posterior compartment and initiates the secretion of dpp at the A-P boundary at the M1 vein. We analyzed the growth patterns of wing disks from distantly related butterflies and moths with dramatically different wing shapes: *Junonia coenia*, *Vanessa cardui*, *Papilio polyxenes, Manduca sexta* and *Actias luna*. The shape and size of the anterior and posterior compartments change throughout growth of the wing disk in a species-specific manner, largely due to differences in the amount of cell proliferation in each compartment. These differences in compartment growth are likely due to evolutionary change in morphogen receptor patterning. Overall, the differences in the relative growth of

each compartment produce evolutionary differences in adult wing shape. Further, we show molecular and cellular evidence for the presence of a novel far posterior A-P boundary at the A1 vein. The presence of this novel boundary is documented in all species studied. We suggest that the existence of the M1 and A1 compartment boundaries has led to increased modularity in Lepidopteran wings, providing a plausible mechanism for the evolution of unique wing shapes.

Phenotypic variation in relation to climatic variation in an evergreen tropical rainforest butterfly

Soumen Mallick[*]*, Freerk Molleman, Oskar Brattstrom, Ullasa Kodandaramaiah, Paul M. Brakefield and Erik van Bergen*

[*]School of Biology, IISER-Thiruvananthapuram, India soumen_mallick@iisertvm.ac.in

Seasonal environments select for divergent traits to which short-lived animals may respond by evolving seasonal phenotypic plasticity: using environmental cues to develop the better fitting adult phenotype. In tropical environments, many butterflies show strong seasonal phenotypic plasticity where wet season forms have larger more contrasting colored eyespots, smaller body size and broader transversal bands. Some butterfly species use temperature to predict rainfall, and so develop the appropriate phenotype, but other cues such as host-plant quality and humidity may also be used. We describe seasonal phenotypic plasticity in a humid rainforest by collecting samples of six species of *Bicyclus* for over one year, measuring wing size, colour pattern. The butterflies showed only subtle seasonal phenotypic variation, which appeared mainly cross-correlated with temperature. The seasonal changes in different traits were not synchronous within species suggesting that they are governed by different mechanisms. Some traits showed similar seasonal changes across species, indicating that they use the same conserved mechanism. Overall, our results point at an ancestral mechanism where the higher temperature induces larger eyespots and other wet season traits, a mechanism that is conserved even in environments where high temperature does not predict an approaching wet season and annual temperature variation is modest.

Decoupled reaction norms for two correlated pupal traits in a tropical satyrine butterfly

Harshad Mayekar[*]*and Ullasa Kodandaramaiah*

[*]School of Biology, IISER-Thiruvananthapuram, India harshadecoevo@gmail.com

Pupation substrate induced pupal colour plasticity is known in the tropical satyrine butterfly *Mycalesis mineus*. Green pupae are formed predominantly under leaves while brown pupae exclusively on off-leaf substrates. It is unknown whether pupation substrate influences pupal traits other than colour. Here we report a lesser known pupal trait, ‘pupal spots’, to exhibit a norm of reaction in response to pupation substrate. Pupal spots are markings found in pairs on the abdominal segments of *M. mineus* pupae. We hypothesize that these spots may function as distractive markings against predators. Off-leaf substrates such as stem and soil

are more heterogeneous than leaves, and hence brown pupae were expected to have larger spots than green pupae. Moreover, if off-leaf substrates are associated with larger spot size, off-leaf formed green and brown pupae should have comparable spots. We checked for variation in the relative spot size across pupal colour and pupation substrates. As predicted, green pupae had smaller spot size than brown pupae. Off-leaf formed green pupae had larger spots than green pupae on leaves, but smaller than brown pupae. While pupation substrate influences pupal colour, both pupation substrate and pupal colour affect pupal spot size. Thus both traits could share a common developmental pathway which needs further investigation.

Symposium: Ecological Immunology in Butterflies
Organizers: **Anne Duplouy and Marjo Saastamoinen**

How does a butterfly embryo cope with immune challenges?

Nora Braak[*]*, Melanie Gibbs, Oskar Brattstrom and Casper J. Breuker*

[*]Oxford Brookes University, UK 15058609@brookes.ac.uk

The egg stage is one of the most vulnerable stages in the life-cycle of many insects. Eggs are deposited at a site chosen by their mother, after which they are exposed to the elements without an option to relocate, and thus have to rely on other ways to protect themselves against environmental stressors, such as desiccation, toxic compounds and pathogens. The majority of winged insects, including butterflies, develop a protective sheet of cells surrounding the embryo during early embryogenesis. This sheet of epithelial cells is called the serosa. Furthermore, the serosa also produces a chitin-rich membrane called the serosal cuticle that may offer further protection. It has been suggested that the serosa may protect embryos against desiccation, environmental toxins and even to mount an immune response. Recently we have shown that serosa formation in butterflies is highly divergent, but little known about which genes are upregulated in the butterfly serosa in response to a challenge, and thus its functional repertoire. Here we will present the first results of serosal functioning in the butterfly *Bicyclus anynana*, including gene expression data (e.g. transcriptome and in-situ hybridisation data) after an immune challenge during early embryogenesis.

Adaptive changes in immune genes in two butterflies, *Pieris napi* and *Pararge aegeria*

Christopher Wheat

Stockholm University, Sweden chris.wheat@zoologi.su.se

Insects rely on their innate immune system to successfully mediate complex interactions with their microbiota, as well as the microbes present in the environment. Previous work has shown that components of the canonical immune gene repertoire evolve rapidly and have

evolutionary characteristics originating from interactions with fast-evolving microorganisms. Although these interactions are likely to vary among populations, there is a poor understanding of the microevolutionary dynamics of immune genes, especially in non-Dipteran insects. Here we use the full set of canonical insect immune genes to investigate microevolutionary dynamics acting on these genes between and among populations by comparing three allopatric populations of the Green Veined White butterfly, *Pieris napi* (Linné; Lepidoptera, Pieridae). Immune genes showed increased genetic diversity compared to genes from the rest of the genome and various functional categories exhibited different types of signatures of selection, at different evolutionary scales, presenting a complex pattern of selection dynamics. Signatures of balancing selection were identified in 10 genes, and 17 genes appear to be under positive selection. Genes involved with the cellular arm of the immune response as well as the Toll pathway appear to be enriched among our outlier loci, regardless of functional category. This suggests that the targets of selection might focus upon an entire pathway, more than on functional subsets across pathways. Our microevolutionary results are similar to previously observed macroevolutionary patterns from diverse taxa, suggesting that either the immune system is robust to dramatic differences in life history and microbial communities, or that diverse microbes exert similar selection pressures.

Signatures of Selection on Immunity-related Genes across Monarch Butterfly Populations

*Wen-Hao Tan**, *Andrew J. Mongue, James R. Walters, Jacobus C. de Roode and Nicole M. Gerardo*

*Emory University, USA wtan4@emory.edu

Immunity-related genes that function in cellular and humoral responses are assumed to be under strong selection given their importance in defending against infectious diseases. However, patterns of selection may differ between different components of the immune system, and the strength of selection may vary across populations facing different parasite pressures. Furthermore, the environment in which the host and parasite are embedded can shape defense evolution. Specifically, availability of anti-parasitic resources could relax selection on immune genes. Monarch butterflies (*Danaus plexippus*), which feed on toxic milkweed plants (*Asclepias spp.*) as larvae, are distributed worldwide. The milkweed species on which larvae feed strongly affects monarch resistance against a specialist parasite (*Ophryocyctis elektroscirrha*), with resistance positively correlating with milkweed toxicity. Monarch populations vary in parasite prevalence and in the milkweed species with which they associate. In this study, we examine signatures of selection on sequences of 102 immunity-related genes in four functional classes (recognition, signaling, modulation, and effector) from 37 monarch individuals across four populations. We test if different functional classes exhibit different signatures of selection compared to the genomic background and discuss these patterns of selection in relation to what is known about disease prevalence and host plant availability across these populations.

Defensive symbiosis between bacteria and moths

Galarza Juan[*]*, Murphy Liam, Burdfield-Steel Emily, Nissinen Riita, Rojas Bibiana and Mappes Johanna*

[*]University of Jyvaskyla, Finland juan.galarza@jyu.fi

Microorganisms are ubiquitous in insects. It is estimated that 70% of species host one or more microorganismal symbiont. From a genomic perspective, the acquisition of an endosymbiont may be viewed as a meta-mutation in the host genome since the genetic repertoire of its bacterial partner, including all its metabolic pathways, is incorporated at once. The potential role of symbionts in their hosts inter- specific interactions, such as predator-prey, remains largely unexplored, particularly in wild populations. Here, we address this issue, in the context of chemically defended species, which may rely on bacterial symbionts for their defence production. The aposematic wood tiger moth (*Arctia plantaginis*) releases malodorous drops form its cervical gland when attacked. Characterization of the fluids by GCMS revealed that carboxylic acids and pyrazines as the main components. Pyrazines can be synthesized by some bacteria and fungi during their primary metabolism and have been reported to be a warning odour component of several Lepidopterans against avian predators. However, the protective association between pyrazine- producing bacteria and moths has never been investigated. Here we investigate this association using antibiotics, GCMS, RNA-seq, and predation assays unveiling the role of bacterial communities in the moth's chemical defense.

Effects of plant toxins on monarch butterfly infection, immunity and the gut microbiome

Jacobus de Roode

Emory University, USA jderood@emory.edu

Parasites pose major threats to their hosts' fitness, and hosts can protect themselves against infection through innate immune responses, anti-infection behaviors and microbial defenses. Monarch butterflies are specialist feeders of milkweed host plant species, which vary in their toxicity of secondary chemicals called cardenolides. Monarchs are commonly infected with a protozoan parasite, and infected females preferentially lay their eggs on high-cardenolide species of milkweed, which reduce infection in their offspring. While previous work suggests that these cardenolides directly interfere with parasite infection, current studies also indicate that milkweeds alter the monarch gut microbiome, and that the altered microbiome contributes to parasite resistance. I will summarize the anti-parasitic behaviors of monarchs and the role of the microbiome in determining parasite resistance, and also summarize studies on the effects of cardenolides on monarch innate immunity.

Silk properties and overwinter survival in gregarious larvae of the Glanville fritillary butterfly

Anne Duplouy, Guillaume Minard, Meri Lahteenaro, Susu Rytteri and Marjo Saastamoinen*

*University of Helsinki, Finland anne.duplouy@helsinki.fi

All organisms are threatened by encounters with parasites. These antagonistic interactions induce strong selection for efficient escape strategies in the host. The threat is even more important for species with reduced mobility, or for individuals entering long-term immobile periods, such as diapause. Adults of the Glanville fritillary butterfly (*Melitaea cinxia*) reproduce early summer, and caterpillars develop and diapause during the winter in silk-nests that maintain over 50 individuals each. In the Åland islands, an archipelago between the Finnish and the Swedish coasts, the average over-winter survival of the Glanville fritillary is ~50%. Despite 20 years of intensive monitoring of the population, population dynamics and studies on various aspects of life history of the species, our knowledge on the parasites associated with the butterfly, and factors that influence over-winter survival remain unclear. Here we tested whether the protein composition of the secreted silk, and the species-composition of the silk-associated microbiota may contribute to the survival of diapausing caterpillars in the field. We compared silk and silk glands from wild-collected caterpillars originating from nests, which survived or died the winter in the field, using comparative proteomics, meta barcoding analysis and laboratory experiments.

Immune-development trade-offs in the wood tiger moth *Arctia plantaginis*

Liam Murphy, Juan Galarza and Johanna Mappes*

*University of Jyvaskyla, Finland liam.m.murphy@jyu.fi

The immune system of invertebrates, although differing to that of vertebrates, is still a very complex system and is thought to be a particularly resource expensive investment for invertebrates also. Under high infection risks, upregulation of the immune system may induce a trade-off with other central life-history traits, such as development. However it is not known how insects re-allocate their resources if the immune defences are not required and if they are subsequently down regulated. Larvae of the wood tiger moth (*Arctia plantaginis*) were raised in the laboratory, on an artificial diet, and treated with antibiotics during their development. Their rate of development was recorded throughout their lives. The efficacy of the defence fluids from newly emerged adults on avian predators was tested. Larvae treated with antibiotics presented a development time that was significantly shorter compared to that of control larvae. Females received a greater boost from the antibiotic treatment than males. These differences point towards the trade-off between the larval immune system and their development, and that this is still a major investment for larvae despite being in an environment with far less pathogens than in the wild.

A plant pathogen modulates the effects of secondary metabolites on the performance and immune function of an insect herbivore

Rosa Elena[*]*, Luisa Woestmann, Biere Arjen and Marjo Saastamoinen*

[*]University of Helsinki, Finland elena.rosa@helsinki.fi

The performance of insect herbivores is dependent on the chemical composition of host plants, which can be seriously altered by plant pathogens, in turn modifying insect-plant interactions. We tested whether a plant pathogen can modulate the effects of the plant secondary metabolites iridoid glycosides (IGs) on the performance and immune defense of a specialist insect herbivore. We used as model system the IG-specialized Glanville fritillary butterfly (*Melitaea cinxia*), its host plant *Plantago lanceolata*, and a naturally occurring plant pathogen (powdery mildew). We found that a powdery mildew infection considerably reduced the beneficial effects of dietary IGs on larval growth. Moreover, mildew presence downregulated one component of larval immune response (phenoloxidase activity), indicating a physiological cost of investment in immunity under suboptimal conditions. Yet, feeding on mildew infected leaves caused an upregulation of two immune genes, lysozyme and prophenoloxidase. Our findings indicate that the performance of an insect specialized on IG-rich plants can be clearly modulated by a plant pathogen. Finally, the contrasting effects on insect immunity induced by the powdery mildew (or by the alterations it produced on the host plant) may be interpreted as a maladaptive response to a supposedly harmless plant pathogen.

Symposium: Colourful Stories: The How's and Why's of Butterfly Structural Colours
Organizers: **Anupama Prakash** and **Seah Kwi Shan**

Heliconius iridescence: natural hybrid zones elucidate the evolution and genetics of structural colour

Nicola Nadeau[*]*, Emma Curran, Melanie Brien, Juan Enciso and Andrew Parnell*

[*]University of Sheffield, UK n.nadeau@sheffield.ac.uk

Structural colours are among the brightest and most impressive in nature and are widely used in animal signalling and communication. There is also interest in understanding and replicating them, for example to prevent counterfeiting or reduce reflection on solar panels. However, virtually nothing is known about their genetic basis or how genetic differences translate into the developmental control of colour producing nanostructures. We are using variation in iridescent structural colour within the *Heliconius* butterflies as a system in which to close this gap. Populations of the co-mimetic butterflies *H. erato* and *H. melpomene* on the Western slope of the Andes in Ecuador and Colombia have an iridescent blue colour that is absent from most other populations of these species. We have identified the structures

responsible for producing these colours to be layered ridges on the scale surface, similar to those seen in other iridescent butterflies, such as *Morpho*. We are mapping the genes responsible for the structural colour difference using controlled crosses and the natural hybrid zone between iridescent and matt black subspecies. Analysis of the hybrid zone is also providing information about the selective pressures acting on iridescent colour in these species.

Melanin pathway genes regulate color and morphology of butterfly wing scales

*Yuji Matsuoka*and Antónia Monteiro*

*National University of Singapore, Singapore dbsyuji@nus.edu.sg

The cuticular skeleton of a butterfly wing scale cell serves both as a substrate for the deposition of pigments and as an exquisitely finely-sculpted material responsible for the production of structural colors. While cuticle rigidity and pigmentation depend to a large extent on the end products of a branched biochemical pathway “the melanin pathway” little is known whether genes in this pathway also play a role in the development of specific scale morphologies that might aid in the development of structural colors. Here we first show that male and female *Bicyclus anynana* butterflies display differences in scale size and scale morphology but no differences in scale color. Then we use CRISPR/Cas9 to show that knockout mutations in five genes that function in the melanin pathway, TH, DDC, yellow, ebony, and aaNAT, affect both the fine structure and the coloration of the wing scales. Most dramatically, mutations in yellow led to extra horizontal cuticular laminae on the surface of scales, whereas mutations in DDC led to taller and sheet-like vertical cuticular laminae throughout each scale. We identify some of the first genes affecting the development of scale morphology, and whose regulation and pleiotropic effects may be important in creating, as well as limiting, the diversity of structural as well as pigmentary colors observed in butterflies.

Genetic basis and evolutionary context for structural color shift in the Buckeye butterfly

*Rachel C. Thayer*and Nipam H. Patel*

*Department of Integrative Biology, University of California Berkeley, USA thayerr@berkeley.edu

Structural color is caused by interference of light as it encounters nanoscale physical structures near the surface of an organism. Although structural color is a pervasive phenomenon, the biological production of the nanostructures remains poorly understood. I am using *Junonia coenia*, a lab-tractable butterfly with extreme intraspecific structural color variation, to overcome two significant challenges inherent to interrogating biological structural color. I show that *J. coenia* responded to artificial selection on color by a 50% thickness increase of each scale lamina, thus shifting color from brown to blue. There was no associated change in pigmentation, providing a rare opportunity to genetically parse loci that regulate specifically the structural component of overall wing color. A second advantage is

that *J. coenia* presents a very simple type of structural color, with only one variable dimension. I can therefore compute reliable measurements of the nanostructure from spectra, making it feasible to phenotype large sample sizes quantitatively without electron microscopy. I exploit these advantages to genetically map quantitative trait loci that control nanostructural dimensions. Lastly, I show that this mode of color evolution (i.e. altering the thickness of scale laminae) recapitulates naturally evolved wing color variation throughout the genus *Junonia*.

Open Symposium

Organizers: **Nathan Morehouse** and **Ramprasad Neethiraj**

Coevolution of male and female reproductive proteins in the Cabbage White butterfly, *Pieris rapae*

Nathan, I, Morehouse, Camille, Meslin, Melissa, S, Plakke and Nathan, L, Clark*

*University of Cincinnati, USA colorevolution@uc.edu

Sexual reproduction is a social interface that intertwines the fitness of males and females. As a result, coevolution between the sexes is expected to be a core driver of reproductive evolution. Interactions between male ejaculate proteins and the female reproductive tract are a critical interface for such coevolution. However, although coevolutionary processes are often invoked to explain patterns of diversity in these traits, how such coevolution plays out at the molecular level remains poorly understood. Here, we present the results of a series of genomic, transcriptomic, and functional studies focused on understanding the identities, functions, and coevolution of male and female reproductive proteins in the Cabbage White butterfly, *Pieris rapae*. Male P. *rapae* transfer a large, complex spermatophore to a specialized female reproductive organ, the bursa copulatrix. We describe the evolutionary histories of key spermatophore proteins, and how they provide distinct spermatophore functions (e.g. digestion resistance versus nutrition). We then describe the origins and evolution of relevant female reproductive proteins, including proteases involved in spermatophore digestion. We conclude by describing ongoing work to connect these reproductive proteins in the context of rapid coevolution, including their role in reproductive dysfunctions during incipient speciation.

Evolution of morphologies and associated behaviours: how geometric morphometrics and kinematics can spread light on the evolution of *Morpho* butterflies wings

Camille Le Roy, Ramiro Godoy-Diana, Florian Muijres, Violaine Llaurens and Vincent Debat*

*Association SCIRE, France leroy.camille7@gmail.com

Butterflies display an extreme variation in wing shape associated with a tremendous ecological diversity. Disentangling the role of neutral vs. adaptive processes in wing shape diversification is a great challenge for evolutionary biologists. In the Neotropical genus

Morpho, wing shape divergence has been linked to the role of ecological differences between micro-habitats (understory vs. canopy). However, whether wing shape and flight behaviour have coevolved in response to contrasted selective pressures exerted by these two micro-habitats still remains to be investigated. Here we conducted a field study to quantify flight behaviour between canopy and understory *Morpho* species using multi-camera three-dimensional videography in cages settled in their natural environment. We then precisely quantified wing shape variation of the filmed specimens using geometric morphometrics to investigate the association between wing shape and flight behaviour. Finally, basic flight parameters (i.e. wingbeat frequency, gliding vs. flapping phases duration) obtained through an analysis of flight sequence shot in the wild are compared with behaviour observed in our field cages. Our study uncovers diversified flight behaviours among *Morpho* species, challenging the classical, binary opposition between understory and canopy species. This stress the need to identify selective forces acting on flight performance.

Female butterflies modulate investment in reproduction and flight in response to migration

*Vaishali Bhaumik**and Krushnamegh Kunte*

*National Centre for Biological Sciences, India vaishalib@ncbs.res.in

Migratory species may display striking phenotypic plasticity during individual lifetimes. This may include differential investment in body parts and functions, differential resource use and allocation, and behavioural changes between migratory and non-migratory phases. While migration-related phenotypic changes are well-reported, their underlying mechanisms are usually poorly understood. Here we compare individuals from migratory (reproductive diapause) and non-migratory (reproductive) phases of closely related aposematic butterfly species to study how sexual dimorphism and migratory behaviour underlie significant morphological tradeoffs, and propose a plausible scenario to explain the migration-related phenotypic plasticity observed in females of migratory species. We found that female butterflies invested significantly more in their abdominal mass compared to males irrespective of their migratory phase, and underwent a clear shift in their body mass allocation after the switch from the reproductive diapause phase to the reproductive phase. In reproductive phase, females invested much more in reproductive tissue. This switch occurred as a result of increased abdominal mass (i.e. reproductive tissue mass) without significant reduction in the thoracic mass (i.e. flight muscle mass). Migratory males, however, were not significantly different from non-migratory males in terms of relative investment in flight and reproductive tissues. These patterns were consistent between migratory and non-migratory aposematic species within and across clades. While migratory habits may influence the physiology and behaviour of both sexes, long-distance migration affected female morphology much more markedly compared to that of males. These results show the sex-specific nature of adaptations to migratory behaviour, and suggest that seemingly disparate life-history traits such as aposematism and migration may have similar influences on the lifetime energetic investments of insects.

Allele specific expression reveals that the sex chromosome is overrepresented in a local adaptation phenotype

Ramprasad Neethiraj, Maria Celorio, Soren Nylin and Christopher West Wheat*

*Department of Zoology, Stockholm University, Sweden ramprasad.neethiraj@zoologi.su.se

While changes in gene expression are documented to be more important than coding changes in local adaptation, identifying which among the 100 if not 1000's of differentially expressed genes (DEG) between populations are cis vs. trans regulation changes remains largely unexplored in non-model species. Thus, if we want to advance our understanding of the basis of local adaptation, the identification of which DEG are cis–regulated population changes are needed. Here we assessed allele specific expression (ASE) using hybrids between populations from dramatically different habitats. A male with obligate diapause (Sweden) was crossed to a female with plastic diapause (Spain). Using a split brood design, F1 males and females were reared under diapause or direct conditions (n=3 for each of 4 conditions), we conducted RNA-Seq on 4 different tissues. ASE was observed in 1877 and 2063 genes in directly developing females and males, respectively, whereas 3468 and 2290 genes showed ASE in diapausing females and males, respectively. The excess of ASE in diapausing females suggest that the Z chromosome via its trans-acting factors interacting with cis-enhancer variation in the autosomes, is an important component of local adaptation.

Genome instability in blue butterflies (Lycaenidae)

Petr Nguyen

University of South Bohemia, Czech Republic petr.nguyen@prf.jcu.cz

For their striking karyotype variation (n=10-226), blue butterflies (Lycaenidae) represent an interesting model for investigating a role of chromosomal changes in speciation. Chromosome fragmentation occurred in three clades of the subtribe *Polyommatina*. To identify a source of the karyotype instability, we have been analyzing genomes of its representatives with both stable and fragmented chromosome prints. Cytogenetic analysis in *Polyommatus icarus* (n=23) suggests a presence of sex chromosome-autosome fusion. Notably, karyotype of *P. (Lysandra) coridon* (n=90) revealed that unlike autosomes, the sex chromosomes avoid the fragmentation and correspond to a large chromosome pair previously reported from polyommatine karyotypes. We recovered positive correlation between chromosome numbers and genome sizes suggesting that the genome instability is accompanied by lineage-specific burst of repeats. Genomes of species under study were sequenced to perform a direct analysis of repetitive sequences using the RepeatExplorer pipeline. Comparison of *P. escheri* (n=23) with *P. (Agrodiaetus) fulgens* (n=109) and *P. (A.) ripartii* (n=90) identified two mobile elements highly abundant in the *Agrodietus* species but virtually absent in their outgroup. The elements were classified as the Ty3/Gypsy retrotransposon and the Tc1/Mariner DNA transposon. The latter is a cut-and-paste element and could be thus responsible for chromosome fragmentation observed.

Symposium: Evolution and Diversification of Aposematism and Mimicry Systems

Organizers: **Krushnamegh Kunte** and **Johanna Mappes**

The evolution of hidden colour signals in moths (and other insect groups)

Tom N. Sherrat[*]*, Changku Kang, Reza Zahiri and Karl Loeffler-Henry*

[*]Dept. of Biology, Carleton University, Canada Tom.Sherratt@Carleton.ca

Many insects have evolved cryptic forewings yet display conspicuous hindwings upon approach or contact with a potential predator. This sudden change of contrast may serve to startle predators, but it can also serve to deceive predators while fleeing, making the prey harder to catch and/or difficult to find when they land. Despite the potential benefits, it is unclear why hidden colour displays have evolved in some species but not in others. To address this question, we conducted a comparative phylogenetic analysis of the evolution of contrasting hindwings in Erebidae and Saturniidae (Lepidoptera), as well as a number of other insect groups. After controlling for phylogeny, we found that contrasting hindwings are consistently associated with larger species. To understand why this is so, we presented a remote-controlled robotic moth to groups of black-capped chickadees. This "robomoth"• could quickly reveal hindwings of particular size and colour when birds approached. Our experiments showed that the survivorship advantage to wing opening was highest when the moth was large and its hindwing was conspicuous. Collectively our results indicate that the phylogenetic association is caused by the greater need for a secondary defence in larger species, and the higher deterrence of conspicuous signals in large species.

Evolution of chemical defenses in the genus Heliconius

Llaurens V[*]*, Sculfort O, Chouteau M, Arias M, Dezeure J, Rosser N, de-Silva DL, Meichanetzoglou A, Mahfoud L, Mappes J, Joron M, Elias M and Nay B*

[*]CNRS, France violaine.llaurens@mnhn.fr

Many toxic butterflies display vivid wing colour pattern, used as a warning signal by predators. Evolutionary convergences in these wing colour patterns are frequently observed among distantly related species, creating "mimicry rings" of butterfly species displaying similar colour patterns. The more individuals and/or species share the same warning signal, the more efficiently the signal is recognized by predators, which suffer from the effect of the chemical defenses carried by butterflies. The evolution of chemical defenses within Lepidoptera lineages therefore plays an important role in the evolutionary convergence of mimetic colour pattern. Chemical defenses can be sequestered from food or been neo-synthetized by butterflies, and may exhibit large variations within and among species. In turn, those variations have important consequences on predator's behaviour and on the dynamics of mimetic signal. Here we focus on quantitative and qualitative variations in chemical defences in *Heliconius* butterflies, which mainly rely on cyanogenic glucosides. We investigated variations in cyanide concentration between species belonging to various

mimicry rings and their consequences on predator's learning and rejection behaviour. We then study qualitative variations among species using LC-MS-MS and localize these compounds within butterfly bodies using FT-ICR imaging. Finally we use the Heliconius phylogeny to reconstruct the evolutionary history of chemical defenses in the genus, and specifically test the influence of host-plant specialization and mimetic signal convergence.

Ecological and evolutionary consequences of mimicry in *Ithomiine* butterflies

Marianne Elias, Nicolas Chazot, Melanie McClure, Lisa de Silva, Andrea Freitas, Chris Jiggins, Geoff Gallice, Colin Fontaine and Keith Willmott*

*CNRS – ISYEB, France elias@mnhn.fr

Ithomiine butterflies (Nymphalidae: Ithomiini) form a neotropical tribe of 393 species, all engaged in Mullerian mimicry. While mimicry is thought to be instrumental in the diversification of the well-studied Heliconius butterflies, little is known of the impact of mimicry in the diversification of Ithomiini. Taking advantage of a new phylogeny of Ithomiini (encompassing 87% of the species) and over 15 years of field data collections, we investigate the ecological and evolutionary consequences of mimicry in Ithomiini. We find that shifts in color pattern are statistically associated with speciation events, thus supporting a role of mimicry in the diversification of the tribe. Furthermore, community-level data show that mimicry has a strong structuring effect on local and regional species assemblages, with implications for the evolution of microhabitat, altitudinal and larval hostplant niches. Taken together, our results suggest that mimicry has played a major role during the evolution of ithomiine butterflies, both in terms of diversification and ecology. Several challenges remain ahead of us, such as detecting the genetic bases of color pattern variation, but also understanding a most intriguing puzzle in Ithomiini mimicry: the evolution of wing transparency in a system supposed to favor conspicuousness.

Why do all extraordinary cases of mimicry come from the tropics?

Johanna Mappes

University of Jyväskylä, Finland

Protective mimicry, in particular among lepidopterans, is perhaps the most classic and powerful school-book example of natural selection. At the same time, mimicry is also a highly paradoxical adaptation. Why are some mimics so extraordinary similar and sometimes so poor? Why do we often see polymorphia among müllerian mimics when positive frequency dependent selection should fix the most common signals? And why do all beautiful examples of mimics seem to come from the tropics? I will show some results, both from the laboratory and field, and discuss how alternative prey community, along with the predator community structure, can facilitate or relax selection on mimetic perfection.

Evolutionary assembly in butterfly mimicry rings

Krushnamegh Kunte

National Centre for Biological Sciences, India krushnamegh@ncbs.res.in

Species co-occurrence in ecological communities is thought to be influenced by multiple ecological and evolutionary processes, especially colonization and competition. However, effects of other interspecific interactions and evolutionary relationships are less explored. We examined evolutionary histories of community members and roles of mutualistic and parasitic interactions (Müllerian and Batesian mimicry, respectively) in the assembly of mimetic butterfly communities called mimicry rings in tropical forests of the Western Ghats, India. We found that Mullerian mimics were phylogenetically clustered, sharing aposematic signals due to common ancestry. On the other hand Batesian mimics joined mimicry rings through convergent evolution and random phylogenetic assembly. Since the Western Ghats are a habitat island, we compared species diversity and composition in its mimicry rings with those of habitat mainland to test effects of biogeographic connectivity. The Western Ghats consisted of fewer mimicry rings and an overall smaller number of aposematic species and mimics compared to habitat mainland. The depauperate mimicry rings in the Western Ghats could have resulted from stochastic processes, reflecting their long temporal and spatial isolation and trickling colonization by the mimetic butterfly communities. These results highlight how evolutionary history, biogeographic isolation and stochastic colonization influence the evolutionary assembly and diversity of ecological communities.

Trade-offs between chemical defense and life-history and fitness traits in a *Heliconius* butterfly

*Anniina L. K. Mattila**, *Chris Jiggins, Lisa Gawriyski, Owen McMillan and Marjo Saastamoinen*

*University of Helsinki, Finland anniina.mattila@helsinki.fi

The evolution of the impressive diversity in the wing patterns of tropical *Heliconius* butterflies has been highly influenced by species mimicking each others' toxicity-advertising warning coloration. However, the factors affecting toxicity of these butterflies are not well known. Recent studies particularly stress the need to investigate ecological traits to understand the evolution of toxicity in mimetic species. *Heliconius* butterflies can metabolize and sequester cyanogenic glucoside toxins from their larval host plants, as well as synthesize cyanogens de novo. Processing and synthesizing these toxins is expected to require a significant amount of energy and resources. Therefore, there may be trade-offs between investing in chemical defense vs. other traits, for instance in defending yourself against predation vs. disease, or investing in chemical defense vs. producing more offspring. We are using common-garden experiments to investigate the associations and potential trade-offs between cyanogenic toxicity and life-history and fitness traits in *Heliconius erato*. We focus especially on energetically costly traits such as fecundity, immune defense, metabolic rate, flight morphology and lifespan. The findings will shed light on factors and constraints involved in the evolution of chemical defense in *Heliconius* butterflies, an important evolutionary model system, and other similar species.

The genetics of assortative mating in *Heliconius cydno*

*Erica L. Westerman**, *Nick VanKuren, Darli Massaro, Ayse Tenger-Trolander and Marcus Kronforst*

*University of Arkansas, USA ewesterm@uark.edu

The genetics of assortative mating has long fascinated biologists, however identifying genes responsible for variation in mate selection has proven difficult due to the complexity of the mate selection process. Here we take advantage of next generation sequencing and variation in male preference for a single visual trait in *Heliconius cydno* butterflies to identify genes associated with visual attraction and assortative mating. We first identify the causative gene controlling the visual attractant in *H. cydno* (a white or yellow forewing band), aristaless 1, using GWAS and CRISPR/Cas-9. We then test whether this same gene, or related SNPs, are highly associated with either life-long mate preference or initial male preference for white- or yellow- banded females. We found that neither life-time preference nor initial male preference are highly associated with aristaless 1 (alr-1). However, life-time (but not initial) preference is highly associated with loci adjacent to alr-1. These results suggest that assortative mating may be driven by genetic coupling in *H. cydno*, and that the genes influencing evolutionarily important behaviors such as mate selection may change throughout an individual's lifetime.

The role of pharmacophagy in unpalatability, aposematism and courtship of clearwing butterflies

*McClure M**, *Corentin, C Cau, M, Meichanetsoglou, A, Mahrouche, L, Nay, B, Furtos, A and Elias, M.*

*CNRS-MNHN-ISYEB, France mel_mcclure@hotmail.com

Ithomiini, or clearwing butterflies, numerically dominate Neotropical forests and act as distasteful models in multiple mimicry rings. Except for primitive genera that feed on Apocynaceae whose leaves contain pyrrolizidine alkaloids (PAs), most species have shifted to Solanaceae as hostplants. These butterflies have continued to satisfy their dependence on PAs for defence against predation and as male pheromone precursors by collecting these compounds pharmacophagously from plants they visit. Here we look at the important consequences of obtaining chemical defences as adults for aposematism (signalling unpalatability to predators) and mate recognition. Because younger individuals may have little or no PAs, they may be more palatable than older individuals, and this is likely to have important implications for aposematism. To test this, PA content was quantified for four different species and chicks were used as model predators to measure unpalatability and speed of avoidance learning for individuals caught in the field, and individuals that we reared

with or without access to PA sources. Male pheromones for one species were also analysed for field-caught males and males reared with and without access to PAs. The evolutionary and ecological role of PA pharmacophagy in unpalatability, aposematism and courtship of clearwing butterflies is discussed.

The unexpected evolution of transparency in aposematic Lepidoptera

*Charline Pinna**, *Malle Vilbert, Melanie Mcclure, Willy, Daney de Marcillac, Stephan, Borensztajn, Florence, Prunier, Nipam Patel, Serge Berthier, Christine, Andraud, Doris Gomez and Marianne Elias*

*CNRS-ISYEB, France charline.pinna1@edu.mnhn.fr

Lepidoptera wing typically display opaque and often colourful patterns, due to scales that cover entirely the transparent chitin-made wing membrane. Such wing patterns are believed to be adaptive and involved in mate choice and antipredator strategies such as aposematism. Locally, different aposematic species converge in wing pattern and form mimicry rings. Surprisingly, mimicry rings may also involve clearwing butterflies. The evolution of transparency among aposematic Lepidoptera raises several questions: 1) How is transparency achieved in different species? 2) Are optical properties of transparent zones convergent within mimicry rings? To address these questions, we performed a phylogenetic comparative study on 64 species belonging to six Lepidoptera families and 11 mimicry rings. We characterised wing structures by SEM imaging and we quantified transparency in the UV-visible range by light transmission. We found that transparency strongly depends on wing coverage by scales, but also on nanostructures present on the membrane. We found no evidence that optical properties of transparent patches are convergent among comimetic species. We conclude that transparency can be achieved by multiple means that may lead to different optical properties. We discuss possible roles of transparency in aposematic butterflies in light of our results and highlight perspectives for future research.

Evolution of flight morphology in butterfly mimicry rings

*Dipendra Nath Basu**, *Vaishali Bhaumik and Krushnamegh Kunte*

*National Centre for Biological Sciences, India dipendran@ncbs.res.in

Prey-predator interactions in mimicry systems are strong drivers for the evolution of adaptive traits in butterflies. Most studies on mimicry have focussed on the ecology and evolution of visual cues used in mimicry. However, selection must act on multiple traits, such as behaviour and associated morphology. In this study, we investigated mimetic resemblance in flight morphology such as wing loading, body-mass distribution and wing shape across mimicry rings in a tropical habitat and in a phylogenetically corrected manner. We observed resemblance in flight parameters including wing beat frequency, manoeuvrability and flight speed between Batesian mimics and their models, which had diverged significantly from their phylogenetic background. We further investigated morphological attributes (body-mass

distribution, wing loading and wing shape) in tropical mimicry rings and their sister species to understand whether the divergence of phenotypes was modulated differently by mimicry and phylogenetic relatedness. Our results indicated presence of a strong phylogenetic signal for all three morphological features, except in a few sex-limited Batesian mimics that showed convergence of these morphological features with their models. Our study thus showed that mimicry differentially affects the evolution of flight morphology and behaviour, with flight behaviour converging on the models in mimetic species while their flight morphology is largely phylogenetically conserved.

Symposium: Notes from the Field: Observations and Hypotheses
Organizers: **David Lohman**

Discovery of the life history of *Papilio demoleus* sthenelinus in the Lesser Sunda Islands

Martin Wiemers[*]*and David J Lohman*

[*]UFZ – Helmholtz Centre for Environmental Research, Halle, Germany martin.wiemers@ufz.de

During the 1990s, invasive SE Asian populations of the Lime Swallowtail *Papilio demoleus* reached the Lesser Sunda Islands (see Morgun & Wiemers 2012), some of which are home to the endemic ssp. sthenelinus, confirmed recently from Rinca Island (Wikipedia 2015). The fate of the endemic population on Flores was unknown until Lambkin (2017) recorded its continued presence on the island near Labuan Bajo. In January 2018, a visit to the Lesser Sunda Islands confirmed the presence of the endemic sthenelinus on the islands of Flores, Komodo and Rinca, as well as the occurrence of invasive populations on Flores and Kanawa Island. For the first time, the life history and foodplants of both the endemic and invasive populations were documented in the Lesser Sunda Islands. Whereas the caterpillars of the endemic sthenelinus exclusively feed on *Cullen gaudichaudianum* (Fabaceae), an endemic species commonly growing in savannahs of the Lesser Sunda Islands, the invasive populations were only found on introduced Citrus (Rutaceae). Endemic and invasive populations occur in sympatry near Labuan Bajo on Flores Island, which raises the question whether they actually belong to different species. Further research is needed on possible hybridization and gene flow between the endemic and invasive populations.

Population biogeography of *Papilio indra* and its larval host plants

Wayne H. Whaley

Utah Valley University, USA wwhaley@uvu.edu

My presentation will be on the population biogeography of *Papilio indra* and it larval host plants. Most North American butterfly enthusiasts consider *Papilio indra* and its subspecies to be the most popular and most sought after swallowtail butterfly of North America. The species consists of 12 described subspecies which are restricted to the Intermountain West region of North America. The subspecies use just 22 plant genera as larval host within the huge family *Apiaceae*, each subspecies linked to one genus. The presentation will illustrate each subspecies with the geographic ranges of each along with the geographic ranges of each subspecies host plants. The distinguishing characteristics of each *P.i.* ssp. will be illustrated along with the distinguishing characteristics of the caterpillar. The larval stages of each P.i. ssp. will also be illustrated. The habitat characteristics of each *P.i.* ssp. will be describes and illustrated. Beautiful photos of each subspecies and their larval host plants will be illustrated. Although *P. indra* has some machaon-like similarities it is considered an outgroup to the *P. machaon* complex. PowerPoint presentation of 100 slides. Author has a website providing general information about the butterfly at: http://research.uvu.edu/whaley/.

Uncertainty about flying conspecifics causes territorial contests of the swallowtail *Papilio machaon*

Tsuyoshi Takeuchi, Shinji Yabuta and Hiroyuki Takasaki*

*Osaka Prefecture University, Japan ttt26715@osakafu-u.ac.jp

Aerial contests of territorial butterflies are difficult to explain on the basis of usual contest models because of butterflies' apparent inability to inflict substantial costs on their opponent. As an alternative, the "erroneous courtship hypothesis" was presented. This hypothesis assumes that territorial butterflies cannot sex the flying conspecifics, and regards the aerial contest of male butterflies as an inevitable same-sex entanglement in their behavioral sequence. To test the robustness of the hypothesis, we investigated the sex recognition abilities of the swallowtail *Papilio machaon*, which is one of the most incompatible species with the erroneous courtship hypothesis as its males perform typical courtship flights to flying females We presented four types of flapping butterfly specimens (fresh male and female, chemicals-removed male and female) to territorial males. They touched fresh female specimens and showed the courtship flight. For the other types of specimens, males rarely showed the courtship flight although they approached or touched them. In addition, males reacted longer to fresh males than to fresh females. These results indicated that although territorial males recognize conspecific flying females by sensing their semiochemicals, they cannot identify flying males, and continue to gather information on them. Thus, the erroneous courtship hypothesis was not disproved.

Some remarkable discoveries of butterflies and diurnal moths in the monsoon tropics of northern Australia

Michael Braby

The Australian National University, Australia michael.braby@anu.edu.au

Between 2005 and 2015 I spent 10 years conducting field work for butterflies and diurnal moths in the Kimberley and 'Top End' in the monsoon tropics of northern Australia, a remote and distinct geographical region renowned internationally for its large and relatively intact savannah landscapes, high biodiversity and strong Indigenous culture. During that period a number of significant discoveries were made, including new species and novel (bizarre) life histories. However, I also rediscovered two species that had not been recorded for over a century: ***Hecatesia sp.*** 'Amata' (Noctuidae: Agaristinae) was previously know only from a single specimen in 1887, and *Ogyris iphis doddi* (Lycaenidae) was known only from a handful of specimens in 1908-09. A brief history of these fascinating species, their ecology, the trials and tribulations of working in remote areas, and the importance of conducting field work to fill basic knowledge gaps will be presented.

Butterfly field observations resulted in evolutionary and taxonomic hypotheses

*Elena Pazhenkova**and Vladimir Lukhtanov*

*St. Petersburg State University, Russia epazhenkova@mail.ru

Field studies and observation often leads to results that cannot be predicted by theory, laboratory research or experiments. During our butterfly survey of Israeli butterflies (2012-2017) several observations were done that initiated further studies and lead to unexpected conclusions. Thus, observation of rare migrants of the Blue pansy (*Junonia orithya*) resulted in a hypothesis about Asian (non-African) origin of these butterflies in Near East. This was later tested through analysis of molecular markers. An attempt to identify a fritillary butterfly observed on slope of Hermon Mts lead not only to a new species detection and description, but also to hypothesis about homoploid hybrid origin of the discovered entity. This hypothesis is now tested using full genome sequencing. Observation of a common species, the dusky meadow brown (Hyponephele lycaon), in two contrasting biotopes lead to discovery of a complex of cryptic species in this group. The role of simple observations in nature is often neglected in science, but often these observations, rather than routine laboratory studies, are the real triggers for startling discoveries.

Specific features of the butterfly fauna of Vietnam with emphasis on biogeography; field surveys 1994-2018

Alexander L. Monastyrskii

Fauna and Flora International Vietnam Programme, Vietnam maack60@hotmail.com

Using a recent classification system based on morphological and molecular studies, 1190 species from 342 genera in 6 families were recorded in Vietnam as of January 2018. There are at least 1280 species-level taxa (species and subspecies) in Vietnam. The Vietnamese butterfly fauna is characterized by high endemism. Approximately 7% of all species are considered to be endemic to the country. Some are restricted to the Indochinese peninsula border and have not yet been found in adjacent areas. The majority of endemic butterfly species range over the isolated mountain massifs of the central Vietnamese Highlands and some centres of endemism in the northern part of the country. Another remarkable characteristic of the fauna are the many examples of paired phenotypic resemblance. Such similarity may be explained as a result of either interactive character of their geographical ranges or as a result of sympatric speciation. Many pairs of different, phenotypically identical species with distinctive genitalia or behaviour are found sympatrically. In addition, the range sizes of Vietnamese butterflies are diverse (nine categories). Configurative ranges (isolated, continuous, disjunct) and conjugated ranges (vicarious, interactive, and mimetic) are a good tool for inferring community assembly processes.

Investigating the worldwide migrations of the Painted Lady butterfly (*Vanessa cardui*) through integrative research

Gerard Talavera, Tomasz Suchan, Clement Bataille, Naomi Pierce and Roger Vila*

*Institute of Evolutionary Biology (CSIC-UPF), Spain gerard.talavera@csic.es

Insect migration is still a poorly understood phenomenon, partly because of the lack of field data and the technical limitations associated with tracking small organisms. The Painted Lady butterfly (*Vanessa cardui*) is an emerging model system for insect migration, movement ecology and evolutionary research. It is the most cosmopolitan of all butterflies and it exhibits what is potentially the widest distributional range of any insect performing large-scale migratory movements. However, little information is known about the species global migratory routes. Here, I will present a multidisciplinary approach to investigate V. cardui seasonal movements and population dynamics, including DNA phylogeography, stable isotope ecology, pollen metabarcoding, ecological niche modelling and field exploration. With a particular focus on the Palearctic-African migratory system, I will describe the latest discoveries showing regular trans-Saharan migrations. These migrations entail astonishing distances of >4000 km, similar to those of some birds, and the findings delineate a new scenario ranking the Afrotropics as a key temporal region in the annual migratory circuit of the species in this part of the world.

Tilapia as a potential standard carrion bait for studies of tropical butterfly communities

Patricio A. Salazar, Ãnderson Medina, Andrea Tapia, Carlos Morochz and Keith Willmott*

*McGuire Center for Lepidoptera and Biodiversity, Florida Museum, UK patoafan@gmail.com

Long-term and large-scale studies of butterfly communities require standard sampling techniques to permit valid comparisons between distant times and places. In the tropics, butterfly traps baited with fermented banana have become a standard technique for studies of community ecology and in butterfly monitoring. Carrion baits are also commonly used, but no single type of carrion bait has yet been adopted as standard. We therefore assessed the efficiency of Tilapia (*Oreochromis spp.*), a freshwater fish cultivated across the tropics, as a potentially suitable standard carrion bait, due to its widespread availability, even far from rivers or sea, and its usually low cost. We conducted an experiment in western Ecuador to test whether Tilapia attracts the same butterfly abundance, diversity and species composition as other commonly used carrion baits and to compare its sampling spectrum to that of the banana standard. We found that all carrion baits recorded higher butterfly abundance than banana but found no evidence of them sampling a higher species diversity. Species composition was very similar among carrion baits, but significantly different from that sampled with banana. Overall, our results indicate that Tilapia could be a suitable standard carrion bait for long-term and large-scale studies of tropical butterfly communities.

The potential for lightweight unmanned aerial vehicles to revolutionise butterfly research

Robin Curtis

University of Exeter, UK r.curtis@exeter.ac.uk

The accurate monitoring of long term trends in butterfly population dynamics is essential to understand changes in the environment and for the development of an effective conservation strategy. We monitor two closely related species, the Glanville and Marsh fritillaries, by conducting annual larval web surveys. The Glanville fritillary is one of the rarest butterflies in the U.K., and has been monitored annually since the mid 1990's. The Marsh fritillary is one of the most rapidly declining species in the U.K., and web counts have been conducted on the Lizard, Cornwall for the last 5 years. Originally, web localities were recorded using handheld GPS and paper maps, but we are now incorporating drones, thermal cameras and high accuracy DGPS in our research to answer fundamental questions on population dynamics and the potential impact of climate change. We describe how using modern technology can help in explaining patterns in distribution and population dynamics and reveal whether it is it now possible to monitor butterfly populations using drones.

Symposium: Diversification, Speciation and Biogeography in the Tropics
Organizers: **Nicolas Chazot** and **Pavel Matos**

Speciation and genome evolution in cryptic wood-white (Leptidea) butterflies.

Venkat Talla, Christer Wiklund, Magne Friberg, Vlad Dinca, Roger Vila and Niclas Backström[*]

[*]Uppsala University, Sweden, niclas.backstrom@ebc.uu.se

Wood-white (Leptidea) butterflies are similar in external appearance but can be separated based on genital morphology, behavior, host-plant preference and karyotype structure. In addition, populations within species sometimes show considerable variation in chromosome numbers (eg. 2n = 56-106 in *L. sinapis*). With the aim to understand both proximate and ultimate mechanisms underlying lineage differentiation, we assembled the genome of the wood white (*L. sinapis*) and re-sequenced the genomes of population samples of two closely related species (*L. reali* and *L. juvernica*) to quantify regional variation in genetic diversity and differentiation. Our data show that the genome size of Leptidea is significantly larger than most previously sequenced Lepidoptera species, a consequence of a comparatively recent burst in activity of specific transposable elements. The genomic data also indicate a deeper divergence than previous estimates based on mitochondrial DNA, and we found no evidence of post-divergence gene-flow between any species pair, despite observations of hybridization in nature. We found that genomic differentiation is heterogeneous between species, with limited evidence of parallelism and significant differentiation associated with chromosome number changes within *L. sinapis*. Both the genome-scan approach and a complementary expression analysis revealed candidate loci for lineage specific adaptations.

From subtribal classification to cryptic species delimitation: progress in clarifying the systematics of the diverse *Euptychiina* butterflies (Nymphalidae, Satyrinae)

Shinichi Nakahara, Keith R. Willmott, Denise Tan and Marianne Espeland*

*University of Florida, USA, kwillmott@flmnh.ufl.edu

The mostly small, brown butterflies of the satyrine subtribe *Euptychiina* occur throughout temperate and tropical North and South America, in fields, grasslands and savannah, as well as rain forests and cloud forests. Although often common, many species are difficult or impossible to identify, due to wing patterns that may vary substantially within species but minimally between species, morphological homogeneity, a poor understanding of species relationships and generic limits, and, historically, a lack of taxonomic work. The last couple of decades, however, have seen a renaissance in euptychiine research, with work on the higher-level phylogenetics as well as generic revisions, by dozens of collaborators in Europe and the Americas. The Lamas (2004) checklist recognized 400 species (described and undescribed) in 40 genera; currently, we estimate the group to contain 542 species in 72 genera, with 76 species and 7 genera described since 2004. An estimated 137 species and 24 genera are still to be described. Focusing mainly on work done over the last five years at the McGuire Center for Lepidoptera and Biodiversity, one of several institutions currently collaborating in euptychiine research, we summarize some of our ongoing molecular phylogenetic and taxonomic revisionary research.

Patterns of biodiversity of butterflies and diurnal moths in the monsoon tropics of northern Australia

M.F. Braby, D.C. Franklin, D. Bisa, M.R. Williams, C.L. Bishop and A.A.E. Williams*

*The Australian National University, Australia michael.braby@anu.edu.au

Northern Australia is a vast region that includes the Kimberley, the Top End, the Gulf Country and Cape York Peninsula. Collectively, these areas comprise the Australian Monsoon Tropics biome, a distinct geographical region renowned internationally for its large and relatively intact natural landscapes, high biodiversity and strong Indigenous culture. We compiled a comprehensive inventory of the butterflies and diurnal moths of the western portion of this region to determine the composition, distribution, relative abundance, breeding status and conservation status of the fauna. The overall goal was to assess how well the fauna is represented in the conservation reserve system and to identify ‘hotspot’ areas. Such information is vital for conservation management and provides a baseline against which the extent and direction of change can be assessed in future. Our dataset comprising approximately 23,500 records based on field observations (61%), museum specimens (28%) and literature (11%) represents 4,352 sites and spans more than 110 years of recording effort. These data indicate that 166 taxa representing 163 species (132 butterflies and 31 diurnal moths) have been recorded from the study region. Broad patterns of species richness, endemism, key threatening processes and priority areas for conservation across the study region will be discussed.

Host repertoire evolution and diversification of butterflies

*Mariana P Braga**, *Soren Nylin and Niklas Janz*

*Stockholm University, Sweden mariana.braga@zoologi.su.se

The diversification of herbivorous insects is often explained by the parasitic use of host plants, as plants represent a great diversity of resources. In their classical paper, Ehrlich and Raven assumed that a trait that allows an individual organism to explore a novel niche also promotes diversification, as the new niche would equate to a new adaptive zone, relatively free of competition. However, the mechanism connecting the increase in individual fitness to an increase in cladogenesis was not specified. Even though this gap in how micro- and macroevolution are connected resulted in a range of proposed mechanisms, little consensus has been achieved. One of the main standing questions is the relative frequency of host colonisations with and without changes in host specificity (host range expansion vs. host shifting), and their importance for diversification. To tackle this problem, we combined various methods to study the origins of macroevolutionary patterns of host use and parasite diversity. From the mechanisms that allow a parasite to expand its host range (coupled with expansion of overall niche breadth), to the effects that evolution of host-parasite interactions has on diversification. For example, we are developing a Bayesian inference method to reconstruct the host repertoire of parasite lineages (that can be applied to other ecological interactions). We also suggest a new approach that combines network and phylogenetic analyses to assess how insect-plant interactions changed through evolutionary time, and how that relates to host-associated diversification. Based on changes in network structure through time, we propose the integration of alternative explanations to the evolution of insect-plant interactions into a single process. With the recent recognition that host-parasite and herbivorous insect-plant systems have much in common, this unified explanation likely has important implications to other host-parasite systems, extending far beyond the studied butterfly families.

Molecular systematics of Haeterini (Nymphalidae: Satyrinae): disagreements between the multispecies coalescent model and traditional taxonomic approaches

Pavel Matos-Maravi[*]*, Carla Penz and Niklas Wahlberg*

[*]University of Gothenburg, Sweden pavel.matos@bioenv.gu.se

The multispecies coalescence model (MSC) is one of the most widely used approaches to delimit species, but recent modeling studies questioned its efficacy. Empirical information and a direct comparison of MSC to current taxonomic knowledge are needed to shed light on the efficacy of MSC as compared to traditional taxonomic approaches. We studied the molecular systematics of clearwing-satyr butterflies (Satyrinae: Haeterini) and compared species delimitations using MSC (implemented in STACEY in BEAST2) to the current taxonomy of the group. Our molecular dataset included 80% of the described species and 50% of the described subspecies, which were DNA-sequenced for 1 mitochondrial and 5 nuclear loci. We found that in many cases the MSC delimitation was congruent with traditionally viewed subspecies. Nonetheless, the number of delimited species by MSC was two-fold higher than that by traditional, morphology-based taxonomy. Diversification rate analyses based on MSC vs. taxonomic species trees revealed striking discrepancies: while the taxonomy-based species tree fit a constant-rate diversification model, the MSC-based species tree suggested a net diversification rate increase in the Pleistocene. This finding suggests that estimation of diversification rates using standard methods is highly susceptible to the definition of species followed by individual authors.

Out of (species) bounds: genome-wide SNPs uncover phylogenetic inconsistencies in the genus *Speyeria*

Erin O Campbell[*]*, Ed Gage, Randy Gage and Felix AH Sperling*

[*]University of Alberta, Canada eocampbe@ualberta.ca

Species delimitation in the North American genus *Speyeria* has been complicated by high variability in wing patterning within species, close evolutionary relationships between species, and the use of only a few molecular markers to infer phylogenetic relationships. Genome-wide SNPs have been useful for elucidating relationships in similarly difficult-to-characterize systems and thus represent a promising approach for clarifying species dynamics within *Speyeria*. We present a phylogenetic comparison between DNA barcoding and genomic SNPs to evaluate incongruities between markers. Our results reveal a history of

complex interactions likely marked by incomplete lineage sorting, secondary contact and introgression, each of which has implications for species delimitation in this group.

Diversification of Nymphalidae butterflies: assembling the "big picture"

*Nicolas Chazot***and Niklas Wahlberg*

*University of Lund, Sweden chazotn@gmail.com

We assembled a dataset of 2868 species of Nymphalidae, 50% of the extant diversity, including 11 gene fragments. The tree was time-calibrated and assembled using a tree grafting procedure combined with secondary calibrations from a recent time-calibrated tree of all butterflies. Nymphalidae originated 83 Ma, but we did not find any signal of a mass extinction event at the K-Pg boundary. We found however, nine major shifts of diversification dynamics across clades. Combining our tree to extant species distributions we estimated that Nymphalidae originated and started diversifying in the Eastern Paleartic region before repeatedly colonizing the different tropical regions. We statistically assessed the departure of the mean diversification rate in 6 biogeographic regions from a random distribution of the diversification rate. Only Neartic and Palearctic regions did not differ from randomized diversification rates, while all tropical regions departed from it. Despite being by far the most species-rich, the Netropical region exhibits the lowest diversification rate, probably thanks to repeated colonisations and the lowest mean extinction rate. By contrast, mean extinction rate was high in tropical Asia while a very wide range of diversification rates with extremely fast and recent radiations characterized the Afrotropical region.

Modes of reproductive isolation depend on geography in a pair of *Heliconius* butterflies.

Neil Rosser

Harvard University, USA neil.rosser@york.ac.uk

Identifying the traits that create reproductive isolation and the geographic context of divergence are fundamental tasks in speciation research. *Heliconius elevatus* and *Heliconius pardalinus* are closely related butterflies with strikingly different wing colour patterns. In the majority tree from genomic data, *H. elevatus* and *H. pardalinus* butleri from sympatric populations in the wet forests of the Peruvian Amazon are sister taxa, but with *H. pardalinus* rendered paraphyletic by the allopatric race *H. pardalinus* sergestus from dry forests in the Peruvian Andes. Resequenced genomes show that *H. elevatus* and *H. pardalinus* butleri are nearly panmictic, with FST ≈ 0 across 98% of the genome, punctuated

by ~20 narrow islands with high FST. Nevertheless, the two differ in multiple traits involved in prezygotic isolation (e.g. male colour pattern preference and sex pheromones), and exhibit strong assortative mating. In contrast, genomic differentiation between *H. pardalinus* sergestus and *H. pardalinus* butleri is uniformly high without clear "islands of divergence", yet they differ little in prezygotic isolating traits and mate freely in captivity. However, while *H. elevatus* and *H. pardalinus* butleri are completely interfertile once mated, female F1 hybrids of crosses between *H. pardalinus* sergestus and *H. pardlinus* butleri/*H.elevatus* are sterile. Range overlap, divergent ecology, strong sexual isolation, inter-fertility and reinforcement in *H. elevatus* and *H. pardalinus* butleri are most parsimoniously explained by ecologically driven speciation with gene flow. In contrast, *H. pardalinus* butleri and *H. pardalinus* sergestus appear to have diverged in geographic isolation, with drift or habitat adaptations producing hybrid incompatibilities. Our results show how the order in which different forms of reproductive isolation evolve may depend on geography.

Skipper anchored phylogenomics: Progress toward a comprehensive evolutionary tree for an enigmatic family of butterflies

*Emmanuel FA Toussaint**, *Jesse W Breinholt, Andrew D Warren, Masaya Yago, Kiyoshi Mariyama, Marianne Espeland, Leslie Reis, Robert Guralnick, Naomi E Pierce, David J Lohman and Akito Y Kawahara*

*Florida Museum of Natural History, USA emmanuel.touss1@gmail.com

Butterflies (Papilionoidea) are perhaps the most charismatic insect lineage, yet phylogenetic relationships among them remain incompletely studied and controversial. This is especially true for the skippers (Hesperiidae), one of the most species-rich and poorly studied butterfly families. To shed light on the evolution of this clade, we sequenced nearly 400 loci using Anchored Hybrid Enrichment (AHE) and sampled all tribes and more than 120 genera of skippers to produce a robust phylogenetic backbone for the family. We then used a different AHE probe set to capture legacy genes frequently used in previous phylogenetic studies on butterfly systematics to enhance the taxonomic breadth of the skipper tree of life and allow the use of previously sequenced taxa. Our maximum-likelihood inferences converge on a novel, robust phylogenetic hypothesis for skippers. These results enhance understanding of the evolution of one of the most species-rich butterfly families and will allow in the long term addressing key questions relative to host-plant use, body-size evolution and biogeographic range evolution in this enigmatic group of butterflies.

Host-plant and biogeographical patterns of *Choristoneura* Lederer (Lepidoptera: Tortricidae)

Giovanny Fagua

Pontificia Universidad Javeriana, Colombia fagua@javeriana.edu.co

Species of *Choristoneura* are well known for pest species as the spruce budworm or the oblique banded leafroller; unfortunately, the knowledge about herbivory patterns of the clade have been biased by the higher amount of information generated on pest species. Using maximum parsimony and Bayesian methods, I generated ancestral character state reconstructions of ancestral hostplant families recorded for species of the clade; then I associated this information with available biogeographic patterns. The genus, with a Holarctic origin 23 million years ago (Ma), diversified in the Palearctic region but suffered a major divergence during the Langhian (Miocene), when a Nearctic clade diverged from the Palearctic clade. Later, 13 Ma, synchronous cladogenetic events related to specialization in herbivory divided both, the Nearctic and the Palearctic clades, into coniferophagous and polyphagous lineages. Ancestral character state reconstructions showed a transition from polyphagous herbivores to moth species with a higher specialization on their host-plant species, principally restricted to conifers or rosids. This change was associated with the collapse of boreotropical forest and its replacement by the current biomes.

"ButterflyNet" An integrative framework for comparative biology

David Lohman

City College of New York, USA dlohman@ccny.cuny.edu

ButterflyNet aims to reconstruct a molecular phylogeny of most butterfly species and aggregate species trait and distribution data. The phylogenies and databases from this project will be freely available through the ButterflyNet Dashboard (butterflynet.org) to enable regional and global comparative studies that are currently not feasible, and a YouTube channel provides outreach aimed at teachers and hobbyists (tinyurl.com/bflynet). Most insects, including butterflies, can be regarded as specialized parasites or parasitoids because they consume a single individual of another species, and the taxonomic host range of an insect species is typically limited to a small set of related host taxa. This differs markedly from most vertebrates and plants, which have broader diets and/or less specialized requirements. The majority of published macroecological and macroevolutionary studies focus on vertebrates and plants, in part because large datasets are available for analysis, but their results may not pertain to the vast majority of (insect) life on Earth. By focusing on the best-known, mega diverse insect group, butterflies, we aim to provide tools for large-scale

analyses of a species-rich, globally distributed insect group. The core researchers collaborate with over four dozen butterfly scientists from across the globe, and welcome partnerships with anyone interested in contributing.

Population genetics of cryptic European wood-white butterflies.

*Venkat Talla**, *Vlad Dinca, Roger Vila, Magne Friberg, Christer Wiklund and Niclas Backstrom.*

*Uppsala University, Sweden Venkat.Talla@ebc.uu.se

European Wood-white butterflies (Leptidea) are similar in external appearance but can be separated based on genital morphology, behavior, host-plant preference and karyotype structure. In addition, populations within species sometimes show significant variation in chromosome numbers (e.g. $2n = 56$ 110 in *L. sinapis*). We obtained a high quality genome assembly by sequencing a Swedish *L. sinapis* larva obtained from four generations of full-sib inbreeding and used population samples from six different populations representing three closely related species of the cryptic complex to study patterns of genomic differentiation between these species and model their demographic histories. We found that genomic differentiation was highly heterogeneous in all comparisons between the three species and populations within the species. With no evidence of parallelism and the genomic data indicated deeper divergence times than previous estimates based on mitochondrial DNA. There was no indication of post-divergence gene-flow between any species pair. We also detected significant intraspecific differentiation between specific populations of both *L. sinapis* and *L. juvernica*. In *L. sinapis* the highest level of differentiation was observed between populations with distinct karyotypes. In addition, *L. sinapis* populations seems to have undergone a considerable expansion recently while the two other species showed little to moderate variation in historical population size. The overall nucleotide diversity of the wood whites is low compared to other butterfly species and *L. juvernica* was found to have the lowest nucleotide diversity. We have identified positive correlation between genome wide genetic differentiation (FST) and absolute divergence (DXY) between all three species pointing out to some evidence for divergent selection between these three species. The functions of the genes harboring non-synonymous substitutions between species are mostly involved in cellular and metabolic process. The results provide information on the speciation history in general and how differentiation processes affect genome evolution in lineages that are recently diverged.

Evaluating the Drivers of Afrotropical Biodiversity; Speciation and Dispersal of a Satyrine Butterfly group in a Paleoclimatic Pendulum

*Kwaku Aduse-Poku**, *Erik van Bergen, Oskar Brattström, Szabolcs Sáfián, Steve C. Collins, David J. Lohman, Niklas Wahlberg and Paul M. Brakefield*

*City College of New York, USA kadusepoku@yahoo.com

Compared to other regions, the evolution history of biodiversity in Africa remains among the least studied. With over 100 recognised species occurring in a wide range of habitats across the Afrotropics, the satyrinae butterfly genus, *Bicyclus* presents an excellent opportunity for understanding fundamental processes that spur species diversification and faunal distributional patterns in Africa. We explore the evolutionary history of *Bicyclus* within a phylogenetic framework, reconstructed using 10 molecular markers and 95% of the recognised species. The genus is inferred to have originated during the mid-Miocene from ancestors that were distributed in the Congolian rainforests in Central Africa. However, climatic drying that appears to have adapted to this Miocene climatic and vegetation change and radiated into the largest group of modern *Bicyclus* species. The other eight main lineages, however, only began to diversify later (8-4 Ma), when climatic conditions returned to more favourable warmer and wetter conditions. The results reveal evidence of paleoclimatic patterns and associated vegetation changes on the diversification and biogeography of fauna on the African continent. characterised the mid- and late-Miocene is implicated to have fragmented ancestral populations, resulting in at least nine major clades that diverged between 18-16Ma. Only one of these lineages.

Treading the Wallacean shortfall for an endangered, iconic and in-demand tropical butterfly (*Teinopalpus aureus*)

Shuang Xing, Tsun Fung Au, Wenda Cheng, Felix Landry Yuan, Pauline Dufour, Lien Van Vu, Fenghai Jia, Min Wang and Timothy C Bonebrake.

The University of Hong Kong, Hong Kong xingshuangbjfu@gmail.com

The insufficiency in geographical distribution information for species, described as the Wallacean shortfall, has been a common problem among insects and could be critical for threatened species. Due to its rarity and striking appearance, the Golden Kaiser-i-Hind (*Teinopalpus aureus*), is popular in the trade but its conservation status remains unclear. By collecting distribution information for *T. aureus*, we applied species distribution models to evaluate the vulnerability of the species under multiple threats with the consideration of conservation efforts across countries. We found that *T. aureus* is predicted to lose 56% of its suitable climatic habitat and its mean suitable elevation would shift about 274 m up with future climate changes. In addition, most suitable habitats for *T. aureus* are currently

outside protected areas and suitable habitats within protected areas will continue to shrink with changing climates. Conservation efforts in protecting *T. aureus* vary across countries, with the highest proportion of suitable habitats protected in Laos and the strictest policy against poaching and trade of the species in China. Our work highlights the importance of data collection for threatened butterflies and emphasizes the challenges in cross-border conservation for in-demand species under global change impacts.

Does historical admixture predict patterns of introgression in a contemporary hybrid zone? A comparison of recent and ancient admixture in *Lycaeides* butterflies

*Samridhi Chaturvedi**, *Lauren K. Lucas and Zachariah Gompert*

*Utah State University, USA samridhi.chaturvedi@gmail.com

Studies of replicate hybrid zones have found evidence of consistency and variability in patterns of introgression, but these studies generally focus on hybrid zones of a similar age. Whether there is consistency in patterns of introgression over time (at different stages of hybrid zone formation) is less clear. Consistency across time (stages of hybrid zones) could imply consistency in the genetic and ecological factors contributing to reproductive isolation and increased predictability of evolution, and perhaps speciation, in general. Here, we use relatively old admixed populations of *Lycaeides melissa* and *Lycaeides idas* butterflies (admixture occurred about 14,000 ybp) and populations from a recent, active hybrid zone to ask if evolutionary patterns in old admixed populations can predict evolutionary dynamics in the current hybrid zone. We addressed two specific questions a). How well can we predict genetic regions which are most resistant to gene flow in recent active hybrids from patterns of ancestry in the admixed populations? b). How well can we predict genetic regions resistant to introgression in both old and new hybrids from known QTL for ecologically important traits? We generated genotyping-by-sequencing data from approximately 25 populations (*L. melissa*, *L. idas*, and admixed populations) to characterize patterns of ancestry and introgression in the old admixed populations and current hybrid zone. We then compared these patterns among populations and asked whether genetic regions with exceptional ancestry frequencies or reduced introgression co-occurred with QTL or genes known to affect traits that contribute to RI in this system (e.g., diapause, host plant preference/performance, or wing pattern).

Using genome-wide representation and *Wolbachia* screening to understand high intraspecific levels of mitochondrial DNA divergence

Vlad Dinca[*]*, Kyung Min Lee, Roger Vila, and Marko Mutanen*

[*]University of Oulu, Finland vlad.e.dinca@gmail.com

The increasing use of molecular data in the study of global biodiversity is revealing unexpected levels of genetic differentiation in numerous species. Most such cases are based on mitochondrial DNA, in large part fuelled by the advent of DNA barcoding, but the interpretation of such patterns is subject to debate. *Melitaea didyma*, a widespread Palaearctic butterfly, stands out as one of the most striking cases: 11 partially sympatric mitochondrial lineages have been reported, displaying levels of mitochondrial DNA (mtDNA) divergence of up to 7.4%. We DNA barcoded 93 specimens of *M. didyma* ranging from Morocco to eastern Kazakhstan and detected ten mtDNA lineages. Wolbachia screening revealed the presence of three strains, two found in single individuals and the third showing high infection rate (72%) in one of the mtDNA lineages. The 93 specimens were further analysed using double-digest RAD sequencing (ddRADseq). The ddRADseq data (22353 loci, 143201 SNPs) revealed five main allopatric lineages recovered as species by STRUCTURE and Bayes factor delimitation analyses. Some of the lineages displayed limited levels of gene flow and showed partial match with the mitochondrial pattern. Certain discrepancies appeared correlated with the presence of *Wolbachia*, suggesting the possibility of mtDNA introgression mediated by *Wolbachia*.

Symposium: Global Citizen Science Monitoring of Butterflies: Building a Globally Engaged Community for Science, Education and Conservation

Organizers: Leslie Ries and Vijay Barve

The monarch butterfly as a model system for the use of citizen science monitoring data

Leslie Ries

Georgetown University, USA leslie.ries@georgetown.edu

The monarch butterfly may well be the single most monitored species in the world. In the US, volunteers spend up to 36,000 hours each year participating in programs designed to collect data on several mechanistic aspects of the monarch life cycle. Monarch-focused monitoring includes counting overwintering roosts, to tracking migration, and the growth and development of juveniles. This is in addition to a roughly equal amount of time doing general butterfly surveys across North America (US and Canada), which also captures information about monarchs (and the 750 other species of North American butterflies). Citizen science data on monarchs has supported up to 2/3rd of the population and conservation studies over the past 20 years. Further, the detailed, spatiotemporally replicated data on population processes is being used to support a framework for future predictions of monarch population dynamics based on a suite of global climate models. The case of the monarch butterfly illustrates the power of using citizen science monitoring networks to capture detailed mechanistic processes and not just distributional observations, and thus has the potential to transform scenario-based modeling for species that are amenable to this level of citizen engagement.

Global Protocols for Butterfly Monitoring

Doug Taron

Peggy Notebaert Nature Museum USA, dtaron@naturemuseum.org

Because of their relative ease of identification and the degree to which their numbers and species diversity are ecologically informative, butterflies have been the subjects of numerous new and longstanding citizen science activities. Many such programs have used the Pollard survey technique, developed in England in the late 1970s. Programs employing the technique are now active in many European countries, as well as in many of the United States. This technique has proved especially useful in detecting long-term population trends. Published data analyses have documented population trends for individual species, groups of species, and butterflies in general. While extremely useful in open, temperate environments, Pollard surveys can be difficult to deploy in tropical forests. A bait trapping protocol can provide a useful alternative in such settings.

A citizen science butterfly monitoring programme in India: Temporal dynamics of butterfly assemblages in a changing urban landscape

Suman Attiwilli[*]*, Rohit Girotra and Krushnamegh Kunte*

[*]National Centre for Biological Sciences, India sumana@ncbs.res.in

We here report on an ongoing long-term population monitoring programme of a butterfly assemblage, a first of its kind citizen science initiative in tropical areas, undertaken by a team of citizen scientists from India. This experiment in citizen science, set within a fast-changing urban landscape, uses time-constrained counts every two weeks, and has resulted in a total of 56,505 counts of 132 species over a span of six years (March 2012 – February 2018). We quantified temporal trends in diversity using a suite of metrics, in order to capture changes in both its α- and β- components. Both α- and β-diversity metrics showed stark seasonal fluctuations. Furthermore, both α- and β-diversity showed significant trends over six years, but only when considering species' presence-absence, ignoring abundances: a) α-diversity: Species richness showed a positive trend, while Simpson's diversity, which takes into account relative abundances, did not show any trend, b) β-diversity: Jaccard dissimilarity increased significantly (of which, species' turnover accounted for 75%; species' additions/eliminations the rest 25%), while Bray-Curtis, a quantitative index of dissimilarity did not show a trend.

These results highlight the efficacy of citizen science, and time-constrained counts, a method possibly better suited for tropical landscapes, in providing high quality data for detecting biodiversity patterns.

Butterflies and Moths of India…Inspiring Citizen Science

*Sanjay Sondhi*and Krushnamegh Kunte*

*Titli trust, India sanjay.sondhi1@gmail.com

The Indian Foundation of Butterflies inspires citizen science in India through internet-based peer-reviewed websites on butterflies and moths. The butterfly website (http://www.ifoundbutterflies.org), started in 2010, covers 1,007 species, and has 48,000 images contributed by 680 individuals. The moth website (http://www.mothsofindia.org), started in 2015, covers 880 species and has over 3,000 images contributed by 106 individuals. The websites also have significant natural history information including life cycles, larval hosts plants, distributions, flight periods, identification keys, bibliography and up-to-date references. We cover the beginnings of the websites, the progress made in the last decade, along with key outcomes. More than 15 new Indian butterfly records, 10 re-discoveries as well as numerous new records and range extensions for moths have been announced on the websites. The websites have recently been expanded to include other taxa such as odonates, cicadas, amphibians, reptiles and birds. Numerous partnering organisations and individuals support the websites on a voluntary basis making them the largest repository on Indian Lepidoptera. Biodiversity marathons and biodiversity meets are conducted to continue to involve citizens in generating scientific data. Plans are being developed to increase website functionality, apps are being developed to enhance ease of data collection and ongoing awareness and outreach programs will result in generation of more meaningful, scientific data.

Symposium: Forgaging Ecology of Butterflies
Organizer: Anuj Jain

Plant resources and disturbance effects on butterfly communities in urban parks

Timothy C. Bonebrake

The University of Hong Kong, Hong Kong tbone@hku.hk

Urban parks can be home to high levels of biodiversity in cities worldwide. However, the habitat quality of urban parks and the long-term prospects for population viability in urban areas are unclear. Focusing on studies in Los Angeles and Hong Kong, I will review on-going research in my lab surveying the factors that determine presence and persistence of butterfly

species in urban green spaces. In Los Angeles we found that rare species can be found in large urban parks and that the abundance of larval host plants is a primary determinant of persistence (absence or rarity of larval host plants is associated with extirpation). In Hong Kong we found that floral resources affected the presence of common species in small urban parks but no environmental factors had impacts on the presence of rare species. Disturbance likely limits the persistence and habitat suitability of rare species in Hong Kong urban parks. In total, the results of these studies work reveal that park size, plant resources and disturbance all structure the communities of butterflies in urban parks and that understanding the life history and foraging ecology of species are key for effective management and conservation.

Host plant preference and performance across life stages in the Glanville fritillary butterfly (*Melitaea cinxia*)

*Ana L. Salgado*and Marjo Saastamoinen*

*University of Helsinki, Finland ana.salgadomaldonado@helsinki.fi

Environmental conditions shape the nutritional landscape of phytophagous insects by changing host plant quantity and quality. In holometabolous insects, the larval stage is crucial for the acquisition of nutrients for development but also survival and fitness during adulthood. Nutritional requirements, as well as the possibility of moving between different host plant individuals, may depend on the developmental stage. For example, in many butterflies, the small and sessile larvae are largely dependent on their mother's oviposition choice in their food choice. We used the *Melitaea cinxia* to test life history responses to feeding on drought exposed host plants, to determine if the observed responses were stage-dependent, and to assess whether post-diapause larvae and adult females show a host plant preference that enhances their own or their offspring performance, respectively. While the performance of post-diapause larvae was increased when feeding on drought exposed host plants, the opposite was the case in pre-diapause larvae. Food choice tests showed that post-diapause larvae fed on control host plants increased their preference for drought treated host, suggesting diet adjustment to enhance their development and survival. Mothers, on the other hand, demonstrated a preference towards control host plants that enhanced the survival of the pre-diapause larvae.

Switching between resistance and tolerance: impact of drought stress on defense strategies of tomato

Po-An Lin, Sulav Paudel, Amin Afzal, Nancy L. Shedd and Gary W. Felton*

*Pennsylvania State University, USA pbl5066@psu.edu

Long-term resource availability affects the evolution of intrinsic defense strategies of plants against herbivores. However, whether similar tradeoffs exist in plant defense strategies against herbivores under short-term resource limitation is largely unknown. Using water

limitation as a short-term resource limiting treatment, we found that severe drought stress increased the resistance of tomato against two caterpillar species with different host range (generalist, *Helicoverpa zea*; specialist, *Manduca sexta*). Increasing severity of drought increased the quantities of three groups of primary metabolites (carbohydrate, lipid, protein), and the activity levels of two defense proteins (trypsin protease inhibitor, polyphenol oxidase) in tomato leaves at different locations. Contrary to the increase in resistance upon water limitation, the regrowth capacity (tolerance to tissue loss) of tomato decline as the drought severity increase. The simultaneous increase in resistance and reduction in tolerance upon drought stress suggests that short-term water limitation might lead to trade-offs between resistance and tolerance against herbivores in an individual plant. Resource limitation not only influenced the evolution of intrinsic defense strategies of plant species, but also short-term trade-offs between resistance and tolerance.

Symposium: Sensory Ecology of Butterflies and Moths
Organizers: Emilie Dion

Early-exposure to new sex pheromone blend alters mate preference in female butterflies and in their offspring

Emilie Dion[*], Li Xian Pui, Suriya Narayanan and Antónia Monteiro*

[*]National University of Singapore, Singapore dbsdejp@nus.edu.sg

Insects use species-specific sex pheromone blends to attract members of the opposite sex who express the corresponding molecular receptors. Given this lock and key mechanism used for species identification and mate choice, it is currently not well understood how pheromone blends or receptor systems evolve. One possibility is that insects develop preferences for new sex pheromone blends via the process of learning, and that these learned preferences may be passed on to the next generation. We tested these hypotheses by exposing newly emerged *Bicyclus anynana* female butterflies to either wild type or to modified male sex pheromone blends. A few days later, we scored female mating outcome in a choice trial involving both male types. We also assessed the mating outcome of naïve offspring of females that underwent distinct odor learning trials to test for a potential inheritance of learned odor preferences. Naïve (parental) females mated preferentially with Wt-blend males, but females pre-exposed to new blends either shifted their preference to new-blend males, or mated equally with males of either blend type; the response depending on the new blend they were introduced to. Naïve daughters of females who were exposed to new-blend males behaved similarly to their experienced mothers. We demonstrate that females are able to learn preferences for novel pheromone blends in response to a short social experience, and pass that learned preference down to the next generation. This suggests that learning can be a key factor in the evolution of sex pheromone blend recognition and in chemosensory speciation.

Hybrid speciation and adaptive radiation along an elevational gradient in an alpine butterfly

Laurence Després[*], *Thibaut Capblancq and Gabriel Neve*

[*]University Grenoble Alpes, France laurence.despres@univ-grenoble-alpes.fr

Speciation with gene flow is more common than previously thought, but the mechanisms by which phenotypic divergence and reproductive isolation arise are poorly understood. Furthermore, the role of hybridization in species diversification and adaptive radiation is debated. I will first highlight the role of hybridization in the diversification of *Coenonympha* butterflies along altitudinal gradients in the Alps (4 lineages), and discuss differential patterns of genomic introgression in contact zones. The hybrid lineage presents an intermediate morphology between the parental species, while its climatic niche is more similar to the alpine species *C. gardetta*. Unexpectedly, although the hybrid genome is mostly constituted of the lowland species genome (70% *C. arcania* and 30% *C. gardetta*), introgression rates in contact zones were much higher between the hybrid lineage *C. macromma* and *C. gardetta* (2 alpine species) than between *C. macromma* and *C. arcania* (alpine and lowland species respectively). Interestingly, both alpine species emit volatile compounds (Octadecanal, Octadecanol and Eicosanal) that are absent in the lowland species, suggesting pre-mating isolation mechanisms between the alpine and lowland species through olfactory cues. Evidence for local adaptation to cold temperature was found by measuring higher warming-up rate (thorax temperature in controlled conditions) with increasing elevation in the lowland and hybrid species. The alpine species *C. gardetta* has the highest warm-up rate, suggesting that temperature is a main driver of adaptive radiation in this butterfly alpine species complex.

Hidden UV wing patterns and evolution of visual genes in diurnal and nocturnal Lepidoptera.

Yash, Sondhi[*], *Jamie Theobald and Akito Kawahara*

[*]Florida International University, yashsondhi@gmail.com

Lepidoptera usually have three to five functional opsins that are responsible for detecting different wavelengths of light and are essential for colour vision. Nocturnal and diurnal Lepidoptera have vastly different conditions in which their visual systems function, both in the amount of background illumination as well as spectral properties of the visual stimuli they need to detect. This results in different selective pressures on their visual genes and the nature of selection (purifying/diversifying) acting on different opsins can be used to infer their relative importance for day and night vision. We use a dataset of 10 nocturnal and diurnal Lepidoptera transcriptomes and perform phylogenetically informed annotation to identify visual opsins. We model rates of evolution of opsins and compare this to opsin evolution across arthropods. We detect multiple opsin duplications and deletions as well as different rates of sequence evolution between various opsins. UV and blue opsins have significantly different rates of selection between nocturnal and diurnal taxa. We use protein models to detect the location of residues that are under purifying or diversifying selection.

These results corroborate recent studies that show that diurnal Lepidoptera seem to rely more heavily on cryptic UV-based signals than nocturnal Lepidoptera.

Acknowledgements

Cultural Dance: Ambika Kurbet, Avinanda Banerjee, Oindrila Bhattacharjee, Ritusree Biswas.

Butterfly Philately Exhibit:

Indian Postal Service: My Post Counter
Release of special postal cover for BOB 2018 conference

Butterfly stamps: Karnataka Philatelic Society

Butterfly and moth poster: Designers and painters from Srishti School of Art, Design and Technology and NCBS (Minoti Asher and Swati H.A.)

Butterfly frames: Paresh Churi

ncbs

IISER
THIRUVANANTHAPURAM

TITLI TRUST
Conserving nature
Protecting the environment

KARNATAKA PHILATELIC SOCIETY
BANGALORE
FOUNDED 1975

SRISHTI
School of Art, Design and Technology