

**PhD meeting in animal evolutionary biology  
(Berlin – Dresden – Prague – Bayreuth)**

**Svatý Jan pod Skalou**

**2-4 March, 2018**

**PRESENTATION ABSTRACTS**

# KEYNOTE LECTURES

## **Insect immune strategies: resisting, tolerating and remembering infections**

**Sophie Armitage**<sup>1,2</sup>, Megan Kutzer<sup>3</sup>, Joachim Kurtz<sup>2</sup>

<sup>1</sup> Freie Universität Berlin, Institute of Biology, Berlin, Germany

<sup>2</sup> Institute for Evolution and Biodiversity, University of Münster, Germany

<sup>3</sup> IST Austria, Klosterneuburg, Austria

Invertebrate immune priming describes when an individual's initial exposure to a pathogen confers enhanced protection, e.g. increased survival, upon a second exposure. It is thought that priming improves host resistance to a pathogen, which would mean that compared to a non-primed host, a primed host has a lower pathogen load after a second exposure. However, the majority of studies have assessed survival, and not resistance, after priming. Theory predicts that priming could also enhance tolerance. This would mean that primed hosts would be better at reducing the negative fitness consequences of an infection than non-primed hosts. The possibility that priming affects tolerance has not to date been tested, but it is important because resistant individuals are not necessarily the fittest. I will present data from our studies exploring whether priming is mediated by resistance and/or tolerance and whether it is dependent upon host genotype or is a more generalisable phenomenon.

## **Molecular evolution in avian immune defence: fitting pieces of a jigsaw puzzle**

**Michal Vinkler**<sup>1</sup>

<sup>1</sup> Department of Zoology, Charles University, Prague

Various genes involved in immune function are selected for optimal direct molecular interaction with pathogen structures. Apart from genes of the Major histocompatibility complex (MHC), this is typical for innate immune Pattern recognition receptors that are responsible for pathogen detection. Toll-like receptors (TLRs) form an essential class of Pattern recognition receptors that plays a key role in the first line of vertebrate innate immune defence against various pathogens. Avian TLR family comprises in its original state ten receptors, where each receptor homodimer or heterodimer is capable of recognizing a distinct set of microbe-associated molecular patterns. In my talk I will show the variation in avian TLRs on interspecific as well as intraspecific level (both gene content and sequence variation), provide evidence for positive selection at certain sites and discuss predicted functional effects of this variation. The investigation of evolutionary patterns explaining the genetic variability in genes of the TLR family may shed light on selective pressures shaping host-pathogen interactions in general.

# TALKS

## Evolutionary dynamics of the W chromosome in caenophidian snakes

**Barbora Augstenová**, Sofia Mazzoleni, Lukáš Kratochvíl, Michail Rovatsos

Department of Ecology, Faculty of Science, Charles University, Prague, Czech Republic

Caenophidia (assigned also as “advanced snakes”) are traditionally viewed as a group of reptiles with a limited karyotypic variation and stable ZZ/ZW sex chromosomes. The W chromosomes of the caenophidian snakes are heterochromatic, and pioneering studies demonstrated that they are rich in repetitive elements. However, a comparative study of the evolutionary dynamics of the repetitive content of the W chromosome across the whole lineage is missing. Using molecular-cytogenetic techniques, we explored the distribution of four repetitive motifs (microsatellites GATA, GACA, AG and telomeric-like sequences), which are frequently accumulated in differentiated sex chromosomes in vertebrates, in the genomes of 13 species of the caenophidian snakes covering a wide phylogenetic spectrum of the lineage. The results demonstrate a striking variability in the morphology and the repetitive content of the W chromosomes even between closely-related species, which is in contrast to the homology and long-term stability of the gene content of the caenophidian Z chromosome. We uncovered that the tested microsatellite motifs are accumulated on the degenerated, heterochromatic W chromosomes in all tested species of the caenophidian snakes with the exception of the Javan file snake (*Acrochordus javanicus*) representing a basal clade.

## Mating changes the genital microbiome in both sexes of the common bedbug *Cimex lectularius*

**Sara Bellinvia**<sup>1</sup>, Paul R. Johnston<sup>2</sup>, Susan Mbedi<sup>3</sup>, Oliver Otti<sup>1</sup>

<sup>1</sup>Animal Population Ecology, Animal Ecology I, University of Bayreuth, Bayreuth, Germany

<sup>2</sup>Institute for Biology, Free University Berlin, Berlin, Germany

<sup>3</sup>BeGenDiv, Berlin, Germany

Animals have intimate associations with bacteria, found on host surfaces, within and between host cells or associated with specific organ systems, such as reproductive tracts or genitals. To date, little is known about the composition of female and male genital microbiomes and how mating affects them. Further, mating often results in copulatory wounds, even in insects and humans. Such wounds provide an entry port for sexually transmitted microbes. Here we characterised female and male genital microbiomes and investigated the effect of mating on the composition of the genital microbiome, by dissecting and sequencing the microbiome of reproductive organs of virgin and mated common bedbugs *Cimex lectularius* from four different populations on a 16S Illumina platform. We found sex and organ-specific differences in the genital microbiome in all populations. Mating changed the genital microbiomes, possibly due to a partial exchange of already present bacteria with new bacterial species, which was mirrored in the high species turnover between organs from virgin and mated bedbugs and a low nestedness. We predict that such mating-induced changes in the genital

microbiome of males and females challenge host integrity and therefore should select for physiological or immunological mechanisms to manage the resident and transmitted microbes.

### **Termites vs. fungi: how the stage of infection determines the collective response**

**Hannah E. Davis<sup>1</sup>**

<sup>1</sup> Institute of Biology, Freie Universität Berlin, Berlin, Germany

Termites defend their colonies from disease using an array of social behaviours, including allogrooming, cannibalism, and burial. I tested how small groups of eastern subterranean termites (*Reticulitermes flavipes*) deploy these behaviours when presented with a nestmate at different stages of infection with the entomopathogenic fungus *Metarhizium anisopliae*. As expected, the termites groomed pathogen-exposed individuals significantly more than mock-treated controls; however, grooming levels were significantly higher after spore germination than before. Cannibalism became prevalent only after exposed termites became visibly ill, and burial was rarely observed. These results demonstrate that termites employ different strategies depending on the stage of infection. Grooming intensity is linked not only to pathogen presence, but also to germination status, and, given the temporal correlation between cannibalism and visible signs of illness, the host may play a role in triggering its own sacrifice.

### **Evolution of pathogen virulence under social immunity: an experimental approach using entomopathogenic fungi and termite hosts**

**Margy A. Esparza Mora<sup>1</sup>**, Hannah E. Davis<sup>1</sup>, Dino McMahon<sup>2</sup>

<sup>1</sup> Department of Biology, Chemistry, Pharmacy, Institute of Biology, Free University, Berlin

<sup>2</sup> Department of Biology, Bundesanstalt für Materialforschung und -prüfung (BAM), Berlin

Termite species can be a good non-Hymenopteran eusocial model for studying the evolution and function of animal immunity and sociality. Social insects nesting in soil environments are in constant contact with entomopathogens and have evolved a broad range of disease resistance mechanisms within a colony to prevent the occurrence and spread of infectious diseases. In this project, I aim to understand how host social immunity drives the evolution of pathogen virulence, and how different entomopathogenic strategies are defended against by their social insect hosts. Specifically, I will test the following hypotheses: (1) Social immunity is plastic: pathogen identity and life cycle strategy shapes the collective defense of termite colonies. Specially, social immune responses depend on the threat level, which in turn depend on the stage and type of infection. Here, I will use *Metarhizium anisopliae*, with different germination rates to explore how fungus life cycle impacts social immune interactions in *Reticulitermes flavipes*. (2) Selective pressures emerging from well-coordinated social insect immune defenses act as a major driver of evolution of virulence. This will be experimentally

tested using different densities of *R. flavipes* and the application of an individual immune inhibitor: D- $\delta$ -gluconolactone (GDL) to evolve virulence of *M. anisopliae* in a serial passage experiment.

### **Host switching in tropical lice**

**Magdalena Gajdošová<sup>1</sup>**, Oldřich Sychra<sup>2</sup>, Tomáš Albrecht<sup>1,3</sup>, Pavel Munclinger<sup>1</sup>

<sup>1</sup> Department of Zoology, Faculty of Science, Charles University, Czech Republic,

<sup>2</sup> Department of Biology and Wildlife Diseases, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic,

<sup>3</sup> Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Czech Republic

The tropics are unique in many biological aspects including parasitism. However, the studies of host-parasite associations are strongly biased towards temperate regions. We asked whether the tropical environment favours rather parasite cospeciation or host switching. To provide some data on cospeciation in the tropics we analyzed the degree of cospeciation in two genera of tropical passerine lice. Since we found evidence for common host switching in these parasites, we further studied whether feather lice colonized preferentially hosts that share some traits with their original hosts. It turned out that host switching is non-random in these lice and it is constrained by several host traits.

### **Chitinase-Encoding Genes in *Drosophila melanogaster***

**Reda Gamal**

Applied Zoology Group, Faculty of Biology, Technische Universität Dresden

Chitin, a polymer of N-acetyl-D-glucosamine, is the major component of the insect cuticle. While chitin supports various beneficial functions of the cuticle, its rigidity-causing properties hamper growth and morphogenesis. This is overcome by regular moulting cycles during growth. During moulting, chitinolytic enzymes, including chitinases, degrade chitin to low-molecular-weight oligosaccharides. Ten chitinase encoding genes (*Chts*) of *Drosophila melanogaster* were previously identified. In order to elucidate their functions, *Chts* expression profiling was performed during the different developmental stages of *D. melanogaster*. We found a very strong sex-biased expression of *Cht12*, raising the possibility that *Cht12* might be involved in male sexual function. Examining the cellular distribution of *Cht12* using a GFP-tagged mutant revealed that *Cht12* expression localized mainly inside the testes, the seminal vesicle and the ejaculatory bulb of the male reproductive system. Future work will address these possible sexual functions more directly.

## **Interaction of male age and mitonuclear epistasis effects on male reproduction in *Drosophila melanogaster***

**Ruijian Guo**<sup>1</sup>, Ralph Dobler<sup>1</sup>, Damian K. Dowling<sup>2</sup>, Klaus Reinhardt<sup>1</sup>

<sup>1</sup> Applied zoology, Department of biology, TU Dresden, Dresden, Germany

<sup>2</sup> School of Biological Sciences, Monash University, Clayton, Australia

The factors affecting the maintenance of variation of male reproductive success represent a central question in evolution. The variation can be affected by both genetic and environmental factors. We constructed mitonuclear introgression lines of *Drosophila melanogaster* from three geographically different populations and examined males at two ages. This design allowed us to separate the independent effects of mitochondrial and nuclear genomes and male age. Male age and the nuclear genome explained a large proportion of variation in sperm function and male fertility. The mitochondrial genome also had a sizeable effect, expressed primarily through epistatic interaction with the nuclear genome. However, disruption of population-specific coevolved mitonuclear genotypes did not lead to general reductions in performance, as might be predicted under a scenario mitonuclear coadaptation. Most importantly, our findings suggest male fitness is determined by epistatic intergenomic interactions, which are more pronounced in younger males. These results provide us with a deeper understanding of the contribution of mitonuclear epistasis to male reproduction.

## **Predation driving prey trait diversification**

**Bin Jiang**<sup>1</sup>, Dirk J. Mikolajewski<sup>1</sup>, Jens Rolff<sup>1</sup>

<sup>1</sup> Institut für Biologie, Freie Universität Berlin, Berlin, Germany

Predation represents an important selection pressure driving prey trait diversification. Thus, prey evolved a variety of morphological, behavioural and physiological antipredator traits. Here, we present data about antipredator trait diversification from a set of closely related species separating among different predator regimes. The dragonfly genus *Leucorrhinia* comprises five European species that inhabit either lakes being dominated by predatory fish (fish-lakes) or lakes being dominated by invertebrate predators like large dragonfly larvae (dragonfly-lakes). Both predator types differ dramatically in their hunting strategies resulting in different strength and direction of selection comparing fish- and dragonfly-lakes. We found that contrasting to invertebrate-lake species, fish-lake species evolved wider abdomen, bigger abdomen muscle mass, larger branchial chamber size to facilitate high burst-escape speed. At the same time fish-lake species became less active to avoid predators. For providing a strong mechanical support for spines and muscle attachment, fish-lake species evolved thicker endo-cuticle in specific segments. Antipredator traits always functionally related with each other, however, not all traits equally respond to predation pressures. Thus, our results highlight the diversification of antipredator traits and their correlations under predator selection.

**Allele shifts under laboratory selection and local adaptation to low salinity in  
Baltic *Mytilus* hybrids (*M. edulis*, *M. trossulus*)**

Loreen Knöbel<sup>1</sup>, Jennifer Catherine Nascimento Schulze<sup>2\*</sup>, Trystan Sanders<sup>2\*</sup>, Dominique Zeus<sup>1</sup>, Jörn Thomsen<sup>2</sup>, Heiko Stuckas<sup>1+</sup>, Frank Melzner<sup>2+</sup>

<sup>1</sup> Senckenberg Natural History Collections Dresden, Dresden, Germany

<sup>2</sup> GEOMAR Helmholtz Institute for Ocean Research, Kiel, Germany

\* shared first authorship; + shared senior authors

Climate models predict desalination in many marine ecosystems, imposing physiological stress on marine organisms. Species-specific responses are expected, determined by their phenotypic plasticity and adaptation potential over multiple generations. If habitat desalination brings an organism close to the limit of its plasticity window, it may be outcompeted by superior adapted species. Disentangling evolutionary mechanisms underlying adaptation and phenotypic plasticity will enable us to predict species responses to long-term desalination. The low saline Baltic Sea has already been colonised by marine *Mytilus* mussels. Experiments simulating reciprocal translocation of larvae from genetically distinct western Baltic *M. edulis* (16psu, practical salinity unit) and eastern Baltic *M. trossulus* (7psu) demonstrated that Baltic *M. trossulus* larvae suffer from significantly lower mortality at low salinity than Baltic *M. edulis*, indicating local adaptation. In support of this, larvae from a genetically diverse population in the transition zone between both populations (11 psu) exhibited allele shifts towards *M. trossulus* alleles when exposed to desalination. Despite the observed allele shift, interspecific gene flow is still pronounced and may be influencing phenotypic plasticity and/or the adaptive potential. We conclude that although phenotypic plasticity is high, selection under low salinity shapes allelic distribution along the Baltic salinity gradient leading to local adaptation.

**Identification of an XX/XY sex determination system in the Australasian clade  
of chelid turtles (Testudines: Chelidae)**

Sofia Mazzoleni<sup>1</sup>, Barbora Augstenová<sup>1</sup>, Peter Praschag<sup>2</sup>, Uwe Fritz<sup>3</sup>, Markus Auer<sup>3</sup>, Tomáš Protiva<sup>4</sup>, Lukáš Kratochvíl<sup>1</sup>, Michail Rovatsos<sup>1</sup>

<sup>1</sup> Department of Ecology, Charles University, Prague, Czech Republic

<sup>2</sup> Turtle Island, Graz, Austria

<sup>3</sup> Museum of Zoology, Senckenberg Dresden, Germany

<sup>4</sup> landsnails.org, Czech Republic

Cytogenetic traits of turtles from the family Chelidae are poorly explored, and the majority of species are still unstudied. Nevertheless, the presence of sex chromosomes was suggested in few chelid species. Therefore, we decided to expand our knowledge on sex determination of chelids, through classical and molecular cytogenetic methods, such as fluorescence *in situ* hybridization (FISH) with probes specific for repetitive elements (telomeres, microsatellites and rDNA genes) which often accumulate on differentiated sex chromosomes of reptiles, in the Australasian genera *Chelodina* and *Emydura*. We identified an XX/XY sex chromosome system in all analysed species, with strong accumulations of repetitive elements on the Y chromosome. Surprisingly, the sex chromosomes

demonstrate different morphology and repetitive content between *Chelodina* and *Emydura* turtles, despite being closely related. In addition, the sex chromosomes are a pair of microchromosomes in the genus *Chelodina*, but a pair of medium-sized chromosomes in *Emydura*, indicating an independent origin of sex chromosomes in chelids. Our results support the hypothesis that chelids have genotypic and not environmental sex determination. Our future plans are to investigate the genetic content of sex chromosomes in both genera, to explore their homology across a wider phylogenetic scale and to design a molecular sexing method.

### **Susceptibility of *Osmia bicornis* to the honeybee pathogen *Nosema ceranae***

**Uta Müller<sup>1</sup>**

<sup>1</sup> Department of Biology, Zoology/Evolutionary Biology, Free University of Berlin

Wild bees are of outstanding importance for agriculture and some species, like the mason bee *Osmia bicornis*, are even suitable for pollination management. Wild bees are exposed to honeybee pathogens when sharing floral resources. However, pathogen transmission alone does not deliver information about impacts in new hosts. In contrast to honeybees and bumblebees, no infection experiments have been undertaken with solitary species. In order to approach this gap, we fed individuals of *A. mellifera* and *O. bicornis* with spores of the honeybee pathogen *Nosema ceranae*. Our results indicate susceptibility of *O. bicornis* to *N. ceranae*. However, no differences in survival and proportion of infested individuals between the two species were found. Discrepancies to other infection studies on social bees might be due to different pathogen strains. Furthermore we collected wild bees near an infested hive. Pathogen spillover to several species was detected. Our study suggests no higher sensitivity of *O. bicornis* to *N. ceranae* than to honeybees. However, with regard to substantial spillover in the natural environment, more studies are warranted incorporating multiple infections, different strains of *Nosema* and other interacting stressors.

### **Beyond nutrition: host-microbiota interactions drive shifts in the behavioural phenotypes of cockroaches**

**Thorben Sieksmeyer<sup>1,2</sup>, Dino P. McMahon<sup>1,2</sup>**

<sup>1</sup> Department for Materials and Environment, BAM Federal Institute for Materials Research and Testing, Berlin, Germany

<sup>2</sup> Institute of Biology, Free University Berlin, Berlin, Germany

Recent studies have shown that host-microbiota interactions can lead to dramatic changes in host phenotypes, including behaviour. We investigate the causal drivers of microbe-associated shifts in host phenotype by examining how feeding behaviour is modulated by a bacterial infection in an omnivorous cockroach: *Blatta orientalis*. We find that immune challenge by *Pseudomonas entomophila* drives a sharp decline in carbohydrate intake and results in a relative increase in the ratio

of protein to carbohydrate consumed. Additionally, infected cockroaches reduce their overall nutrient intake. We show for the first time that cockroach feeding behaviour is dynamically modulated by a pathogen. In contrast to studies on *Spodoptera* moths, this modulation does not impact any of the immune parameters we measured: it does not affect the abundance of immune related proteins in the hemolymph, hemolymph antimicrobial activity, or survival. An illness-induced anorexia-like response is the most likely cause of the observed phenotype. In another cockroach species *Blattella germanica* we also investigate whether in addition to pathogens, host microbiota may be causally responsible for shifts in host behavioural phenotypes and immune competence. To address this, we conduct food-choice and immune-challenge experiments in germ-free and native gut microbiota-containing cockroaches.

### **Phylogenetic relationships within the *Gobius*-lineage (Gobiidae, Gobiiformes)**

**Tereza Slámová<sup>1</sup>, Radek Šanda<sup>2</sup> & Jasna Vukić<sup>1</sup>**

<sup>1</sup>Department of Ecology, Faculty of Science, Charles University, Prague, Czech Republic

<sup>2</sup>Department of Zoology, National Museum, Prague, Czech Republic

Gobiidae is one of the largest families of teleost fishes. They are distributed worldwide, except Arctic and Antarctic. They inhabit marine, brackish and freshwaters. Mostly, they are small-sized and live inconspicuously on the bottom. Their phylogeny has been studied only partially. In Europe, three independent lineages of gobies exist (*Gobius*-, *Aphia*- and *Pomatoschistus*-lineage), most of the species of these lineages are marine. We performed a multilocus study of the *Gobius*-lineage encompassing the majority of the species. Mitochondrial (cytochrome b and cytochrome c oxidase I) as well as nuclear (rhodopsin and recombination activating gene) markers were used. The results confirm that the genus *Gobius* is not monophyletic. It is divided into two well-supported groups. Also, the genera *Vanneaugobius* and *Padogobius* are polyphyletic. Further, our results revealed existence of cryptic diversity inside genera *Gobius* and *Chromogobius*. The relationships between genera as well as within them are often not resolved well, indicating that the evolutionary events took place in a short time-frame. Finally, the adaptation to freshwaters occurred two times independently in this lineage.

### **The older, the better? How ageing affects plumage coloration, reproductive investment and immunity in great tits (*Parus major*)**

**Martin Těšický<sup>1</sup>, Hana Velová<sup>1</sup>, Jana Svobodová<sup>2</sup>, Petra Bauerová<sup>2</sup>, Tereza Krajzingrová<sup>1</sup>, Oldřich Tomášek<sup>1,3</sup>, Hana Pinkasová<sup>1</sup>, Tomáš Albrecht<sup>1,3</sup> & Michal Vinkler<sup>1</sup>**

<sup>1</sup> Department of Zoology, Charles University, Prague

<sup>2</sup> Department of Ecology, Czech University of Life Science, Prague

<sup>3</sup> Institute of Vertebrate Zoology, Czech Academy of Science, Studenec

During senescence, physiological functions and reproductive success typically decrease whereas the probability of mortality increases. Although symptoms of ageing are well described in humans and model animals, they are still poorly understood in free-living animals. Furthermore, recent studies in birds show that ageing is a complex process and does not necessarily result in the decline of reproduction and in the higher risk of age-associated mortality. The main aim of this contribution is to describe the effect of ageing on sexually selected plumage coloration, immunity, and reproductive investment in great tit (*Parus major*). In 2011-2017, we repeatedly captured 135 individuals of great tits breeding in nest boxes in a small forest at Prague outskirts. As indicated by increased ratio of heterophils to lymphocytes among white blood cells in peripheral blood and higher blood thiol levels in aged birds, old individuals may suffer from higher oxidative stress. Interestingly, middle-aged individuals had the highest yellow chroma and total brightness of the yellow carotenoid-based ornament, and also the biggest clutch size, indicating their high sexual attractiveness and reproductive performance. Our results are consistent with the *Disposable soma theory of ageing* which predicts better immunity and lower investment into reproduction in younger individuals compared to older ones due to the trade-off in energy allocation between reproduction and self-maintenance.

### **Oxidative stress in sperm: effects of storage time, male and gamete age, and metabolic pathway**

**Biz Turnell**<sup>1</sup>, Klaus Reinhardt<sup>2</sup>

<sup>1</sup> Institute of Zoology, Technische Universität Dresden, Dresden, Germany

Sperm age is an understudied trait that may account for much of the currently unexplained across-male variation in fertilization success. As sperm cells metabolize, they produce reactive oxygen species (ROS) that can in excess lead to cellular damage and accelerate the aging process. When sperm is stored for any length of time, both males and female should be under selection to minimize such damage. Here, we use *Drosophila* to investigate the relationships between ROS production and (i) sperm storage duration; (ii) sperm and male age; and (iii) cellular metabolic pathway. We use a range of species that differ in their mating frequency and in the duration of male and female sperm storage to test the prediction that less frequent mating and longer sperm storage is associated with lower ROS production. We also examine the separate and combined effects of male age and gamete age on sperm ROS levels. Finally, we use two metabolic mutants with above-normal and below-normal somatic ROS production to investigate oxidative stress in sperm. Our findings synthesize the field of sexual selection and the theory of cellular senescence, an area of research that has major but as yet largely overlooked implications for sperm use and competition.

### Genetic diversity of the *Pelasgus* species in the Greek Ionian basin

Nuria Viñuela Rodríguez<sup>1</sup>, Radek Šanda<sup>2</sup>, Stamatis Zogaris<sup>3</sup> and Jasna Vukić<sup>1</sup>

<sup>1</sup>Department of Ecology, Faculty of Science, Charles University, Prague, Czech Republic

<sup>2</sup>Department of Zoology, National Museum, Prague, Czech Republic

<sup>3</sup>Hellenic Center for Marine Research, Institute of Marine Biological Resources and Inland Waters, Anavissos, Greece

*Pelagius* is an endemic genus of the southern Balkans, formed by seven recognized species. Four of them occur in the Greek Ionian basin; their exact distribution, relationship between populations and genetic diversity are still not well known. The aim of this work was to analyze such genetic diversity based on mitochondrial and nuclear markers. *Pelagius laconicus* occurs in two river basins in the southern Peloponnesus, and specimens from both drainages share the same haplotypes of cytochrome b. *Pelagius stymphalicus* inhabits most of the Peloponnesus and the mainland region of Aetolia-Acarmania; it has two slightly different groups of cytochrome b haplotypes, corresponding to two geographically separated areas. A species with unclear taxonomic status, provisionally named *Pelagius* cf. *epiroticus*, is present in the rivers flowing into the Amvrakikos Bay, in the uppermost Kalamas River and Zaravina Lake. This lineage was also discovered in two endorheic basins on the Peloponnesus, originally inhabited by *P. stymphalicus*; the analyses of nuclear markers revealed cases of mitonuclear discordance in both localities. *Pelagius thesproticus* occurs from Acheron basin to the south Adriatic slope. Although *Pelagius* species have non-overlapping occurrence area, we observed two drainages inhabited allopatrically by two *Pelagius* species, most probably due to natural processes.

# POSTERS

## Previous microbial exposure (priming) and pathogen infection dynamics

Beatriz Acuña Hidalgo<sup>1</sup>, Sophie Armitage<sup>1</sup>

<sup>1</sup> Institut für Biologie, Freie Universität Berlin

Previous microbial exposure, or “immune priming”, is an example of host phenotypic plasticity in invertebrate immunity: priming confers an individual with increased protection against a secondary encounter with a pathogen. Although its underlying mechanisms are not well understood, evidence for this phenomenon has been found across a large range of taxa, including *Drosophila melanogaster*. Evidence for immune priming has frequently been tested for by assaying survival after the secondary encounter, with the prediction that successfully primed animals will live longer than those that were not primed. Priming could act by reducing the pathogen load (increasing host resistance) after the secondary encounter, which thereby increases survival. However, priming could also theoretically act by increasing host tolerance, i.e., allowing the host to reduce the negative fitness impact of infection. Indeed fitness consequences of immune priming are relatively unexplored. Here, I will present the plan of my doctoral project in which the roles of tolerance and resistance in immune priming will be addressed through experimental infection of *D. melanogaster* with pathogenic bacteria.

## How to avoid pitfalls in sperm viability staining using a sperm stress test

Barbara A. Eckel, Ruijian Guo, Klaus Reinhardt

Applied Zoology, Technische Universität Dresden

Sperm viability (SV), the proportion of live sperm in a sample, is a widely applied measure of sperm quality. However, SV is problematic as a surrogate for sperm quality, for instance as the proportion of live sperm extracted from males appears more related to male than to sperm quality in the sense of the future performance of sperm. We propose an alternative method to assess sperm quality by characterizing the temporal decrease of SV in a stressor medium and illustrate in two species, *Cimex lectularius* and *Drosophila melanogaster*, how some common methodological pitfalls may be circumvented. Our data empirically support the well-known but little-considered facts that (i) non-blind measurements may alter SV and (ii) that SV frequently have non-significant repeatability within one sample. (iii) Cross-sectional sampling of ejaculates showed that this heterogeneity even masked a biological pattern—the sperm stratification within males. We show (iv) that this shortcoming can be overcome by following the temporal decline of SV of a sperm subsample in a stress test. Finally, (v) comparing the staining pattern of sperm between *Cimex* and *Drosophila*, we found that in the latter, the visibility of sperm is substantially delayed when sperm density is high.

### **Determination of the age of bedbugs (*Cimex lectularius*) through pteridines: methodical pitfalls**

**Jana Křemenová<sup>1</sup>**, Tomáš Bartonička<sup>1</sup>, Zdeněk Šimek<sup>2</sup>

<sup>1</sup> Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno,

<sup>2</sup> Research Centre for Toxic Compounds in the Environment, Faculty of Science, Masaryk University, Brno

The individual age for different insect species can be determined by the concentration of pteridines in the eye. The female age and with it the changing ability to keep viable sperm seems to be an important parameter in the study of ecological speciation and reproductive isolation of two host lines of bedbugs (*C. lectularius*) parasitizing humans and bats. So far, no study has been done to determine the age of bedbugs, and there are still some methodological inconsistencies in the age determination. At first we had to choose suitable pteridines because their amount and presence differ among taxa. Furthermore, it was necessary to select the most appropriate extraction medium since its pH significantly affects the quality of the extraction. The next step was to find out whether pteridines are really only in the eye because the preparation of the whole head is much easier and more effective than the eye preparation. Finally, all literature states that pteridines are light-sensitive, so extraction has to be done under very limited light conditions. Preliminary results show that the useable pteridines for identifying the age of the bedbugs are 6-biopterin, isoxanthopterin and leucopterin. For all tested pteridines was found that they are primarily in the eyes and no significant difference between the preparation for light and darkness. Thus, unlike most published procedures, extraction can be performed under the light, which greatly facilitates sample preparation and reduces the error rate.

### **Evolution of holometabolous insects**

**Christin Manthey<sup>1</sup>**, Michael T. Monaghan<sup>2</sup>, Jens Rolff<sup>1</sup>

<sup>1</sup> Institute of Biology, Freie Universität Berlin

<sup>2</sup> Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Berlin

Insects with complete metamorphosis (Holometabola) are the most successful group on earth. Complete metamorphosis radically transforms the insect body, leading to distinct larval and adult stages separated by a pupa. Why the pupa evolved, remains unclear. One barely studied explanation is the decoupling of growth and differentiation in Holometabola. The larva grows and most differentiation occurs in the pupa. By reconstructing the holometabolous 'Eve', I studied the relationship of rapid growth and the evolution of complete metamorphosis. I compiled a dataset on insect growth comprising of 350 species. Due to a wide variety of growth measurements, I calculated growth ratios for 14 of these species. My preliminary results show no correlation between growth and holometabolism. But I could show higher growth rates and higher variance in growth rates in egg size corrected data in Holometabola. However, my dataset builds a well-documented basis to further investigate the decoupling of growth and differentiation in insects with complete metamorphosis.

### Evolution of virulence under frequent multiple infections

**Florent Manzi**<sup>1,2</sup> (supervisors: Justyna Wolinska<sup>1,2</sup>, Frida Ben-Ami<sup>3</sup>)

<sup>1</sup> Dept. of Ecosystem Research, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin

<sup>2</sup> Department of Biology, Chemistry and Pharmacy, Institute of Biology, Freie Universität, Berlin

<sup>3</sup> Department of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University

The amount of damage caused by a parasite to its host – or virulence – is a trait of interest in many epidemiological studies. It is widely assumed that high virulence trades off with transmission at the population scale, as killing the host too fast leaves the parasite with less time to produce and release infective propagules. This non-linear relationship does not take into account complex within-host interactions, such as resource competition, public-good production or interference between co-infecting parasites. In the last few years, theoretical modelling and experimental studies have shifted towards a more realistic, multiple strains-based framework to study the evolution of virulence. Long-term investigations on between-host dynamics and multiple species interactions are still missing, however. During the course of my PhD, I intend to pave the way towards filling this gap, by way of i) long-term parasite evolution experiments in microcosms, coupled with ii) genetic sampling of natural parasite populations, using a *Daphnia*-microparasites system.

### Functional Screening of Mitochondrial Enzymes across 27 Introgression Lines

**Praneetha Sundar Prakash**<sup>1,2</sup>, Susanne Voigt<sup>1</sup>, Klaus Reinhardt<sup>1</sup>

<sup>1</sup>Technische Universität Dresden, Dresden, Saxony, Germany

<sup>2</sup>SASTRA University, Thanjavur, Tamil Nadu, India

Mitochondria's most important function, energy production via oxidative phosphorylation (OXPHOS), depends on tight interactions between products encoded by the mitochondrial as well as the nuclear genome. Introgression lines serve as an analytical tool that helps us further understand the effect of mismatches between mitochondrial and nuclear genomes on these interactions. Twenty-seven introgression lines of *Drosophila melanogaster* were created from three massbred population samples which were derived from three geographically and climatically distinct natural populations: Coffs Harbour in Australia (A), Benin in Africa (B) and Dundas in Canada (C). Three stocks of the following combinations were maintained: **AA**, AB, AC, BA, **BB**, BC, CA, CB and **CC** (The lines in bold are the control lines). When the mitochondrial DNA (mtDNA) of all introgression lines was sequenced, 20 highly differentiated segregating sites (SNPs) were identified in the protein-coding regions, including nonsynonymous as well as synonymous changes. Nonsynonymous substitutions alter the amino acid composition of the protein which in turn may alter the protein conformation and also may affect its functionality. Although synonymous substitutions do not alter the composition of the protein, they may have an effect on the efficiency and accuracy of protein expression and thus might also affect OXPHOS activity. Since, the SNPs are found to be present in genes encoding for components of the

mitochondrial complexes I, IV and V, enzyme activities of these complexes will be evaluated to understand the effects of SNPs on mitochondrial function.

### **Evolving defences: Resistance and tolerance**

**Luís Silva**<sup>1</sup>, Sophie Armitage<sup>1</sup>

<sup>1</sup> Institute of Biology, Freie Universität Berlin, Berlin, Germany

Hosts are able to avoid or limit parasitic infections through two major counter-strategies: via resistance, which inhibits or controls the pathogen; or through tolerance, which limits the negative fitness impact of the pathogen on the host. These strategies are predicted to have distinct evolutionary outcomes. Because resistance has a negative effect on pathogen fitness it will reduce parasite prevalence in the population and lead to negative frequency dependent selection and polymorphisms in the host population. Ultimately, it can lead to increased pathogen virulence and antagonistic co-evolutionary cycles between host and pathogen. In contrast, tolerance does not control pathogen numbers within the host. Thus, tolerance could be neutral or increase parasite prevalence in a population and not select for pathogen counter-adaptations. Although tolerance has broad implications (e.g. medical sciences, livestock breeding), little is known about the impact of evolving damage control at the population level. I will present the plans for my doctoral project in which I will test how selection for host tolerance affects host resistance, and vice-versa, by performing experimental evolution with *Drosophila melanogaster* and a bacterial pathogen.