

114th Annual Meeting of the German Zoological Society (DZG)

12th – 16th of September 2022, Bonn

Abstract Book



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#dzg22

The Phylogenetic Symposium 2022 at the Institute of Evolutionary Biology and Ecology of the University of Bonn



Dear colleagues,

the 62nd Phylogenetic Symposium (“PS2022”) will take place at the University of Bonn from November 18-20, 2022. It will run under the title “Macroevolutionary Dynamics”.

The 2022 symposium aims to bring together experts from the fields of evolutionary modelling, adaptive landscape theory, spatial interaction ecology, tipping point modelling, geometric morphometrics, genotype-phenotype mapping and biomechanics in order to discuss how we can reach a more integrative understanding of the many processes involved in interspecific variation.

Although the particular topic of each symposium is chosen by the chair, it is largely shaped by its speakers, as is reflected by its unique structure: There will be only ~45 min keynote lectures on one and a half days plus up to 30 mins of discussion after each keynote. Speakers are invited to give an overview of their research together with an outline of their ideas for future research directions in the respective research field.

Confirmed speakers:

Claudia Bank (University of Bern, Switzerland)

Tiffany Knight (Helmholtz-Zentrum für Umweltforschung, Germany)

David Labonte (Imperial College London, UK)

John A. Nyakatura (University of Berlin, Germany)

Mihaela Pavličev (University of Vienna, Austria)

P. David Polly (Indiana University, USA)

Moritz Lürig (Lund University)

Emily Rayfield (University of Bristol, UK)

Registration for this symposium is free of charge.

The PS saw a two year pause due to the COVID19 pandemic so that we are more than happy to be able to return to an on-site event now! Although this symposium is free of charge, it is very important to register properly, so that we are able to book tables for the conference dinner, organise the ice breaker etc. **Please register for this meeting until 30.09.2022** by using the registration link on the homepage of the Institute of Evolutionary Biology and Ecology of the University of Bonn. We are then able to keep you in the loop regarding potential COVID regulations and other amendments.

Sincerely,

Alexander Blanke & Carina Edel

Award Winners

Werner-Rathmayer-Preis für eine originelle Arbeit auf dem Gebiet der Zoologie (500 €) - Deutsche Zoologische Gesellschaft e. V.

Preisträger 2022:

Armin Höcherl & Nikolaus Weiland

Schyren-Gymnasium Pfaffenhofen, Pfaffenhofen an der Ilm

Vögel mit Magenproblemen:

Plastik am Himmel – Untersuchung von Speiballen

Nicht nur Seevögel, sondern auch andere Vogelarten in Bayern haben Plastik im Magen. Das fanden Armin Höcherl und Nikolaus Weiland heraus. Sie untersuchten 88 Gewölle von freilebenden Weißstörchen und Greifvögeln, die normalerweise nur Unverdauliches aus der Nahrung enthalten. Die beiden Jungforscher fanden in jedem fünften Speiballen der Störche auch Plastikteilchen. Der Plastikanteil schwankte deutlich, was mit dem jeweiligen Jagdgebiet der Vögel zusammenhängt. Im Labor konnten die beiden darüber hinaus nachweisen, dass das Plastik von Salzsäure nicht zersetzt und im Magen der Störche daher nicht verdaut wird. Die Gewölle der Greifvögel dagegen waren plastikfrei, vermutlich, weil Greifvögel nur selbst erlegte Beute fressen und daher Plastikabfall nicht mit Nahrung verwechseln können.

Karl-Ritter-von-Frisch-Medaille 2022 – Wissenschaftspreis der Deutschen Zoologischen Gesellschaft – dotiert mit 10 000 €

Preisträger sind der Inter-Research Wissenschaftsverlag Oldendorf/Luhe (gegründet von Prof. Dr. Dr. h.c. Otto Kinne) und die Springer-Verlag GmbH Berlin, Heidelberg

Preisträger 2022:

Prof. Dr. Dr. h.c. Thomas C.G. Bosch

Christian-Albrecht-University Kiel, Zoological Institute

Metaorganisms as the new frontier

In the last decade, revolutionary advances have been made from century-old debates about the relative importance of non-pathogenic bacteria. Today we know that animal evolution is intimately linked to the presence of commensal microbes and that individuals are not solitary, homogenous entities but consist of complex communities of many species that likely evolved during a billion years of coexistence. Metaorganisms (hosts and their microbes) are multipartite entities that result from ecological, evolutionary and genetic processes. I will attempt to highlight growing evidence that the innate immune system with its host-specific antimicrobial peptides and rich repertoire of pattern recognition receptors has evolved in response to the need for controlling resident beneficial microbes rather than to defend against invasive pathogens. I will also provide new insight into the original role of the nervous system, and suggest that it emerged to orchestrate multiple functions including host-microbiome interactions. This reorientation has profound implications not only for the understanding of evolutionary biological processes and ecological interactions, but also for our health. I will close with the remark that an understanding of host-microbe associations, and of their evolution and molecular underpinnings, has become the new frontier in zoology.

Public Lecture

Biodiversity and people in the Anthropocene

Katrin Böhning-Gaese

Senckenberg Biodiversity and Climate Research Centre, Frankfurt, Germany

In the Anthropocene, the age of humans, the very same are increasingly overwriting geological and ecological processes in the earth system. This is interlinked with biodiversity loss, climate change and pollution, which are all symptoms of humans overutilizing nature. According to the World Biodiversity Council IPBES, one million of the estimated eight million species on earth are threatened by extinction: We are facing the sixth mass extinction in the history of our planet. Since biodiversity is the existential basis of human life, a decline of biodiversity results in a loss of ecosystem services, which undermines achieving the sustainable development goals. But what can be done? Given the importance of the biodiversity crisis for human well-being, we need a transformation of society at large, i.e. the fundamental system-wide reorganization of politics, economy, civil society and also science. Especially for agricultural landscapes in Germany, where species declines are particularly high, this means three things: First, the greening of agriculture. Second, fundamental changes in agricultural policy, better collaborations and new technologies. Third, changes in consumption and diets towards food coming from biodiverse agricultural production and a much higher share of plant-based foods. If we commit ourselves to quick, deep, sweeping changes including the expansion of protected areas and the promotion of restoration, the decline in biodiversity can still be halted and reversed.

CV Prof. Katrin Böhning-Gaese

Prof. Katrin Böhning-Gaese has been director of the Senckenberg Biodiversity and Climate Research Centre and professor at Goethe University Frankfurt since 2010. She previously held a professorship at the University of Mainz since 2001. The trained biologist focuses on the effects of climate and land-use change on biodiversity, as well on the importance of biodiversity for human well-being. She is speaker of the DFG-funded research unit “The role of nature for human well-being in the Kilimanjaro Social-Ecological System”. Prof. Böhning-Gaese is a member of the German National Academy of Sciences Leopoldina and the Academy of Sciences and Literature Mainz. From 2017 to 2021 she was vice-president of the Leibniz Association. In 2021, she was awarded the German Environmental Award for her outstanding research and her commitment at the interface between science, society and politics.

Keynote Speakers

(Sorted by last name of presenting author)

The mind of a bee

Lars Chittka

Queen Mary University of London, England

Bees have a diverse instinctual repertoire that exceeds in complexity that of most vertebrates. This repertoire allows the social organisation of such feats as the construction of precisely hexagonal honeycombs, an exact climate control system inside their home, the provision of the hive with commodities that must be harvested over a large territory (nectar, pollen, resin, and water), as well as a symbolic communication system that allows them to inform hive members about the location of these commodities. However, the richness of bees' instincts has traditionally been contrasted with the notion that bees' small brains allow little behavioural flexibility and learning behaviour. This view has been entirely overturned in recent years, when it was discovered that bees display abilities such as counting, attention, simple tool use, learning by observation and metacognition (knowing their own knowledge). Thus, some scholars now discuss the possibility of consciousness-like phenomena in the bees. These observations raise the obvious question of how such capacities may be implemented at a neuronal level in the miniature brains of insects. We need to understand the neural circuits, not just the size of brain regions, which underlie these feats. Neural network analyses show that cognitive features found in insects, such as numerosity, attention and categorisation-like processes, may require only very limited neuron numbers. Using computational models of the bees' visual system, we explore whether seemingly advanced cognitive capacities might 'pop out' of the properties of relatively basic neural processes in the insect brain's visual processing area, and their connection with the mushroom bodies, higher order learning centres in the brains of insects.

Why Birds are Smart

Onur Güntürkün

Ruhr-Universität Bochum, Germany

For about a century, bird brains were seen as small, non-cortically organized systems that casted a dim prospect on the cognitive abilities of their beholders. Within the last two decades, this view has changed dramatically. My talk will concentrate on discoveries of the last decade that demonstrate that birds have a prefrontal-like area with identical functional, electrophysiological, neurochemical, and connectional features as the mammalian prefrontal cortex. Similarly, the avian pallium, although topographically and topologically different from the mammalian one, harbors a connectome akin to those of mammals. In addition, avian neuron numbers are not only much higher than expected by brain size, but also mostly allocated to associative areas in corvids. In parallel, birds developed the ability to cut down metabolic demands of their neurons by a factor of three. This not only makes a brain with so many neurons affordable, but may also provide cellular computational properties that are out of reach for mammals. Lastly, birds even developed a sophisticated cortex within their sensory pallial areas – possibly independent from mammals. Thus, avian and mammalian forebrains converged within 315 million years to an astounding degree. Most importantly, these changes happened very likely in convergent manners without relying on common ancestry. Possibly, evolution does not lack creativity but is just facing a severe limitation of degrees of freedom when wiring a vertebrate brain for sophisticated cognition.

The evolution of cranial design and bite force in vertebrates: form, function and evolution

Anthony Herrel

Muséum National d'Histoire Naturelle Paris, France

The transition from water to land was accompanied by major transformations in the vertebrate skull including, amongst others, the evolution of a fleshy tongue and a fusion of many of the skull bones. From this initial relatively mobile skull as seen in amphibians several lineages evolved variably kinetic (lepidosaurs, birds) or largely akinetic (mammals, turtles) skulls. However, the consequences of these changes in skull design on the structure and function of the jaw muscles remains poorly known. Moreover, whether fused akinetic skulls are associated with the evolution of an increase in bite force remains poorly known. In the present talk I will present data on the jaw muscles and bite forces and explore whether major changes skull mobility across clades have gone hand in hand with changes in jaw muscle size and complexity and the consequences thereof on bite force. To do so muscle data of over 300 species of vertebrates and bite force data of over 500 species are analysed. Although previous meta-analyses of bite force have suggested little impact of diet on the evolution of bite force our data suggest otherwise. Finally, I will highlight gaps in our understanding of vertebrate cranial function.

Evolution of gene regulatory factors and their networks - tools and insights into human evolution

Katja Nowick

Freie Universität Berlin, Germany

Humans differ from other primates in various aspects, among them a larger brain and distinct cognitive abilities. Evolutionary changes in gene regulatory factors, such as transcription factors and non-coding RNAs, drive some of these differences. Hence, we investigated which gene regulatory factors have been positively selected on the human lineage. Interestingly, we found several candidates that have been linked to cognitive disorders, arguing that they play an important role in the development and functions of the human brain. We also revealed that transcription factors are interacting more strongly with each other in the human compared to non-human primate brains and identified a human specific module that seems to regulate brain developmental and cognitive processes. To gain more insights into evolutionary consequences of changes in candidate genes, we started investigating target genes and functions of selected transcription factors in cell lines derived from different primates in a comparative way. For ZEB2, we discovered human-specific targets related to neuronal development. With this presentation, I will use the opportunity to talk about our research on human evolution and to present some of the bioinformatics tools we have developed for identifying coding and non-coding genes under selection and for studying evolutionary changes in molecular networks.

Understanding the causes of biodiversity change: experiments, technologies and models

Christoph Scherber

Zoologisches Forschungsmuseum Koenig Bonn, Germany

The world's terrestrial ecosystems currently experience rapid changes in biodiversity that largely happen unexplored and unexplained. For example, local biodiversity loss has been reported for a range of taxa such as mammals, birds or butterflies. Yet, most studies focused on responses of individual taxa or subgroups of taxa, and the drivers of biodiversity change have often remained poorly known. Here, I provide insights from a range of field experiments where primary producer diversity and components of global change have been manipulated to study changes in heterotroph biodiversity. Taken together, these studies show that global change acts on primary producers to affect other trophic levels indirectly in ecological networks. Additionally, I show how novel technologies such as DNA metabarcoding can unravel community structure at unprecedented resolution, opening up new avenues for assessing biodiversity change. I conclude with potential future linkages to ecological modelling that are needed for biodiversity research to become a more predictive field of science.

Evolutionary consequences of parthenogenesis and the loss of sex: Insights from stick insects (and other animals)

Tanja Schwander

University of Lausanne, Switzerland

Transitions from sexual reproduction to female-producing parthenogenesis are expected to be associated with major changes in selection. These changes include the loss of sexual antagonism (because there is no more selection on male phenotypes) and shifts in selection on life-history traits associated with the novel form of reproduction. I will illustrate consequences of such selection changes using *Timema* stick insects, a group with multiple, independent transitions from sexual reproduction to female-producing parthenogenesis.

Invited talks of the Subject Groups

(Sorted by last name of presenting author)

Hiding in plain sight – uncovering the diversity across the phasmatodean tree of life

Sarah Bank-Aubin

University of Göttingen, Germany

Stick and leaf insects (Phasmatodea) are nocturnal herbivorous arthropods characterised by masquerading as plant parts. Despite their fascinating morphological diversity and worldwide tropical distribution, they were only until recently considered one of the lesser-studied insect lineages. This may partly be explained by their unstructured traditional taxonomy as well as a high degree of convergently evolved traits impeding a morphological classification system. However, due to efforts of the past years, molecular phylogenies have begun to shed light on the phylogenetic relationships and raised doubt on the validity of the traditional classification. With the aim to revise the traditional concepts that were mainly based on morphological characters, we inferred the so far largest phasmatodean molecular phylogeny comprising over 1000 stick and leaf insects. By trying to cover a large number of the described species, we discovered a vast amount of hidden and undescribed phasmatodean diversity. This extensive taxon sample highlights those taxa most in need of taxonomic revision and simultaneously provides the optimal framework to discuss systematics and re-assess evolutionary relevant morphological and behavioural traits. For instance, the morphology of the egg has emerged as important characteristic to delimit taxa and even otherwise cryptic species. Furthermore, in combination with geographical data, we reconstructed the historical biogeography of several lineages allowing us to draw conclusions about distribution patterns of an insect group with rather low dispersal capability. The context provided by our phylogenetic analyses offers a sound basis for future work on this diverse lineage from both an evolutionary and a systematics perspective.

Why do we play, and who else does?

Wolf Huetteroth

Universität Leipzig, Germany

Who apart from us is playing? What IS playing? And what is it good for? I will try to give a quick introduction into different forms of play including the wide distribution of play-like behavior among vertebrates, to address two main questions:

Is play-like behavior universal, and if so, what is it good for? Surprisingly, a conclusive answer to this question is still missing. Insects need to tackle the same problems in their life as any other animal (including us): survive and reproduce. So, if play-like behavior has any evolutionary advantage (and it should, since it is connected to serious costs), did insects also exploit this trait? To examine that, I am analyzing flies over several days in an enriched environment, with free access to food and water, and voluntary access to a spinning platform – a carousel. We looked at flies that are blind, proprioceptive impaired, or with different foraging alleles (rover and sitter); some exhibit specific behavioral pattern in the arena, all perform with high individual variability. I will present a working hypothesis for the evolutionary benefit of such play, which postulates that an animal intentionally exposes its proprioceptors (body joint sensors) to external mechanical stimulation, i.e. centripetal force. This ‘intentional exafference’ is then used to challenge and train self-recognition memory (i.e. “self-awareness”) of the organism.

From DNA to ecosystems – The promise of biological archives for biodiversity monitoring

Henrik Krehenwinkel

Trier University, Germany

Detecting the imprints of global environmental change on biological communities is a major challenge for current ecological research. The analysis of the effects of environmental change requires standardized and replicated time series data, which is lacking for most ecosystems and taxa. This lack of community level time series data has become particularly evident with recent reports on global insect decline. Natural history collections are currently receiving attention for their potential to document environmental change, but they usually do not provide community level data. Considering this background, new approaches and data sources are urgently needed to understand historical community change in the Anthropocene. Recent developments in high throughput DNA metabarcoding hold great promise to provide the needed data. The analysis of environmental DNA (eDNA), DNA traces left by organisms in their environment, has particularly revolutionized biodiversity research. eDNA is preserved in many biological archives, making it possible to trace taxonomic changes at unprecedented temporal detail. In my presentation, I will give an overview of our work using eDNA analysis in a large biological archive, the German Environmental Specimen Bank (ESB). In the past three decades, the ESB has compiled biotic samples from various ecosystems of Germany, including marine, limnic and terrestrial habitats. The samples are collected according to highly standardized protocols and permanently stored at ultra-low temperatures, which makes them an ideal source of historical eDNA. I will first highlight our recent work on the reconstruction of temporal changes in German forest insect communities from eDNA traces associated with ESB leaf samples. Then I will discuss other sources of community level eDNA in marine, limnic and terrestrial samples of the ESB and show preliminary results on community change in different taxonomic groups across the tree of life in the past 30 years.

Intrinsic and extrinsic drivers of hypometabolic states in insects

Philipp Lehmann

University of Greifswald, Germany

Diapause is a deep resting stage that facilitates temporal avoidance of unfavourable environmental conditions, and is used by many insects to adapt their life cycle to seasonal variation. Although considerable work has been invested in trying to understand each of the major diapause stages (induction, maintenance and termination), we know very little about the extrinsic and intrinsic drivers of transitions between stages, especially diapause termination. We have investigated these aspects in various insect species, and here I will present three studies using pupae of the butterfly *Pieris napi*. (1) In this species, diapause is associated with both temperature-dependent and -independent processes. While the general diapause phenotype (e.g. suppressed metabolism, increased cold tolerance) is established in a temperature-independent fashion, diapause termination is temperature-dependent and requires a cold signal. (2) The shape of cold-accumulation follows a unimodal nonlinear thermal reaction norm, with optimal rates at winter temperatures. We model this reaction norm as a mirrored version of a typical thermal performance curve and use it to successfully predict diapause termination in multiple fluctuating laboratory temperatures. (3) A putative underlying physiological mechanism of thermal accumulation involves the key developmental hormones prothoracicotropic hormone (PTTH) and ecdysone, whose temperature-dependence we have studied and manipulated during diapause. While PTTH is present throughout diapause in two pairs of neurosecretory cells in the brain, it is absent in the axons and PTTH concentration in the haemolymph is significantly lower during diapause than during post diapause development, indicating that the PTTH pathway is silenced during diapause. At low temperatures, reflecting natural overwintering conditions, diapause termination propensity after ecdysone injection is precocious compared to controls. In stark contrast, at high temperatures reflecting late summer and early autumn conditions, sensitivity to ecdysone does not return. Thus, here we show that PTTH secretion is silenced during diapause maintenance, and additionally, that the low ecdysone sensitivity of early diapause maintenance is lost during termination in a temperature dependent manner. The link between ecdysone sensitivity and low -temperature dependence reveals a putative mechanism of how diapause termination operates in insects that is in line with adaptive expectations for diapause. Further building a model integrating extrinsic (time and temperature) and intrinsic (hormones and metabolites) drivers of diapause progression remains a main goal in our research program.

Flying back in time: molecular switches that have changed insect gastrulation.

Steffen Lemke

Universität Heidelberg, Germany

My laboratory is interested in the molecular basis of evolutionary transitions and innovations. We study such transitions in the context of gastrulation, a critical morphogenetic event that sets up our diverse body plans; without it, animal life in today's complexity probably would not exist. How this program has diversified and repeatedly reorganized itself is a fundamental unanswered question in biology. Our major passion is to address this problem by studying gastrulation in a set of different animal species. This leads us to ask how young embryos of different species generate a first and simple epithelial monolayer, what mechanisms define the properties of such monolayers, and how they fold and deform to establish the body plan. We use a zoo of different fly species to address these questions: flies are easy to keep in the lab, their embryos all follow comparable steps of development, and we can watch the process in real time under the microscope. In comparing non-model fly species with *Drosophila*, we have been able to uncover surprisingly simple switches that change tissue properties or even alter the mode of gastrulation.

Microbiome: a promising avenue to study individual variation in animal behaviour and cognition

Öncü Maraci

Bielefeld University, Germany

The gastrointestinal tracts of the animals are home to diverse and dynamic microbial communities, collectively known as gut microbiota. The discoveries in the last two decades have left no doubt that gut microbiota has mutual connections with several aspects of host physiology, fundamentally altering our understanding of animal biology. Today it is well-established that microbial symbionts are functionally involved in countless processes and adaptations of their hosts, including behaviour and cognition. In the first part of my talk, I will try to elucidate how host and environmental factors interactively shape avian gut microbiota, leading to tremendous individual variation. In the second part of my talk, I will argue how the individual variations in the gut microbiome translate into cognitive and behavioural differences.

Chemical ecology of mate recognition: Signal distinction and variation

Tamara Pokorný

University of Regensburg, Germany

Chemical cues and signals are vital for many, if not most aspects of an insect's life, from finding food to obtaining a mating partner. Recognizing a suitable mate is a prerequisite for reproductive success. The semiochemical compounds and/or their relative amounts usually differ between species, and signal distinction can be further enhanced by temporal or spatial separation. Communication may be initiated already from a distance if volatile compounds are emitted, allowing for mate detection at medium to long ranges. Nonetheless, many species additionally or exclusively rely on chemicals of low volatility for mate recognition at close range. In this context, cuticular lipids play a major role. Complex mixtures of these lipids cover the cuticula of insects, serving as a barrier against desiccation as well as in a communication function. Even closely related species usually exhibit distinct lipid profiles. Close range mate recognition can require a combination of relatively polar lipids and hydrocarbons, which constitute the predominant cuticular lipid class. In several species, though, cuticular hydrocarbons (CHCs) alone elicit courtship behaviour and mating attempts. While CHC profiles are often clearly sex dimorphic, this is not necessarily a prerequisite for their sex pheromone function, as (superficially) similar profiles may be readily distinguished. However, CHC profiles are not invariant, and mate recognition in a case of CHC variation will be addressed in the final part of the talk.

Evolution of mammalian hearing is influenced by major transformations in the chewing biomechanics in the earliest of mammals

Julia A. Schultz

Rheinische Friedrich-Wilhelms-Universität Bonn, Germany

Modern mammals share precise dental occlusion as a defining character. Several evolutionary transformations of the skull and lower jaw were necessary in the mammalian ancestry line to achieve the ability to effectively masticate and also to enhance the sensitivity of hearing. In the past 15 years new fossil findings and novel 3D visualization techniques shed new light on the processes involved in the stunning history of the mammalian ear. Fundamental changes within the features for feeding (such as jaw and teeth) and sensory function (such as sensitive hearing of high-frequency sound) took place, among a whole suite of other evolutionary innovations. During the early mammalian history, mandibular bones with both feeding and hearing functions separated from the jaw to become the true mammalian middle ear bones specialized only for hearing. The three major systems involved are anatomically connected: Lower jaw and dentition, the middle ear and, the inner ear. The mammalian jaw and dentition play a major role in food processing as they function as tools to break down food into smaller pieces. OFA and XROMM analyses show the complexity of the mammalian chewing process beyond simply counting the number of chews. Precise occlusion is characteristic to mammals and the evolution of the large variety of chewing patterns in modern mammals is only possible because of the detachment of the middle ear elements from the jaw in the past. High resolution computed tomography and 3D reconstruction aides to understand the process of incorporating multiple bony elements into the middle ear. A mechanism crucial to increase the hearing sensitivity most notably to high-frequency sounds, a trait that is thought to have benefited the mammalian lineage. The delicate chain of middle ear ossicles is unique to mammals and functions as a sound transmitter for sound waves to reach the inner ear. Here the sound waves are converted into mechanical triggers of nervous stimuli that are perceived as hearing. Traditional histological serial sectioning and 3D animation techniques serve to investigate structural changes in the soft tissue involved in the elongation of the cochlea and innervation pattern necessary for high frequency hearing. In mammalian history the enhancement of chewing performance and hearing ability is inevitably linked to the decoupling of the middle ear bones from the chewing part of the system.

How changing environmental conditions and resource supply affect invertebrate herbivores - Interactive effects on plankton organisms

Erik Sperfeld

Universität Greifswald, Germany

How organisms are affected by changes in environmental conditions or resource supply were and are always key questions in ecological research. This interest can arise from different viewpoints, e.g. whether we are interested in the success or performance of particular species, the transfer of energy and matter through food webs, or in the consequences for biogeochemical cycles. Research of the last century mainly focused on investigating single environmental drivers or single resources to explain animal performance or community responses. However, in the last decades there is increasing awareness that multiple drivers can have interactive effects on organisms or communities and that organisms can be limited by more than one resource simultaneously (co-limitation). Using mainly *Daphnia* sp. (Cladocera, Crustacea) as model organism, I will demonstrate in which ways herbivorous consumers are affected by the availability of multiple nutrients on the individual level. Expanding on the conceptual framework of resource co-limitation, I will show how this concept can be tested across trophic levels to scale up to the community level. I will also show how *Daphnia*, as representative of aquatic ectotherms, responds and adapts to changing temperatures and how the responses are influenced by food quality aspects of their diet. Finally, I will discuss potential effects of thermal variation on the performance of aquatic ectotherms, because previous experimental studies mainly investigated effects under constant temperatures, a situation not found in natural systems.

Hooked on Chaetae - the biological 3D-printers of bristle worms

Ekin Tilic

University of Copenhagen, Denmark

Annelids (bristle worms) produce an array of complex hard structures, like their name-giving bristles, or chaetae. These chitinous extracellular structures display an incredible morphological diversity also reflected in the intricacy of their formation. Chaetogenesis, is a process involving multiform and dynamic microvilli that resembles a biological 3D-printer. In this talk I will present results from past and ongoing research exploring the plasticity of this fascinating system that constructs some of the most characteristic features of Annelida, ranging from the furry feltage that covers the sea mouse, the stinging bristles of fireworms and many more. The intricate cellular machinery that produces chaetae also indicates that there are novel and fine-tuned molecular mechanisms of biomineralization in annelids. This harbours tremendous untapped potential for developing new biomimetic strategies. In most annelids, chaetogenesis is continuous, as chaetae get constantly replaced during the lifetime of an animal, making the ultrastructural characterization of this process often possible even from a single segment of an adult animal. However, the bone-devouring worm *Osedax* is exceptional in that it has a fixed set of chaetae that only emerge once during larval development. This provides a unique opportunity to explore the expression of genes involved in this process. The characterization of chaetogenesis from both a molecular and morphological perspective is key for understanding the evolution of mechanisms that led to the vast diversity of biomineralized structures not just within annelids but also across other metazoa.

Reproductive trait evolution: unravelling the effects of pre- and post-copulatory sexual selection

Cristina Tuni

Ludwig-Maximilians-Universität München, Germany

Sexual selection operates both before and after mating, with evolutionary theory predicting that traits involved in mate acquisition, namely pre-copulatory traits, covary with those involved in fertilization success, namely post-copulatory traits. Based on the nature of these associations, selection acting on traits, such as male ornaments and/or armaments, can potentially constrain or facilitate the evolution of other traits, such as testes and/or sperm phenotype, and *vice-versa*. Using the field cricket *Gryllus bimaculatus* as a model system, my research aims at understanding how pre- and post-copulatory sexual selection shape complex reproductive phenotypes. I will discuss findings from studies uncovering both, phenotypic and genetic correlations among behaviour, morphology, and ejaculate traits, suggesting that traits are integrated at the genetic level. I will further report on a long-term experimental evolution study designed to disentangle the effects of pre- and post-copulatory selective pressures on a range of reproductive traits. The study of male courtship singing, aggressiveness and weaponry (mandibles), testes and sperm traits, will allow understanding whether and which traits diverge in response to altered pre- or post-mating selective pressures, potentially shedding light on correlated evolution and/or evolutionary trade-offs between reproductive traits.

Genetic basis of spider sense organ development

Natascha Turetzek

Ludwig-Maximilians-Universität München, Germany

External sense organs cover the arthropod body, are extremely diverse, and essential as they are required to perceive various sensory modalities and with that interact with and react to the environment. Spiders are of particular interest to study the evolution of sense organs as they set apart by various evolutionary novelties for example silk production and using webs for interspecies communication and prey capture. Signals coming from the web are encoded as vibrations, therefore mechanoreception is probably the most important sense of web building spiders. Consequently, spiders possess a wealth of sense organs all over their body and especially on the appendages. To understand how this great sense organ diversity and spider specific key innovations have evolved it is important to study the genetic basis of sense organ development. Contrasting their great importance and diversity the genetic control of sense organ development is only studied in a handful of arthropod species, mostly insects. Comparative studies are needed to understand which genes play conserved roles during sense organ development and which changes drive diversification. We investigated sense organ candidate genes in spider species from different groups including the spider model species *Parasteatoda tepidariorum*. Interestingly, we found that many candidate genes are most likely not involved in spider sense organ development. The exception are the PAX and ASC transcription factor families, which are expressed in spider sense organ precursor cells during embryogenesis. Interestingly, both gene families underwent several rounds of duplications. We analysed the evolution of these two gene families in chelicerates, with special focus on spiders and examined the expression of all paralogs. We find signals of sub- as well as neofunctionalization, probably important for spider sense organ evolution and diversification, most likely connected with spider specific abilities and behaviors like web building.

Neural circuitry for hormonal release and maternal behavior in mice

Silvana Valtcheva

Uniklinik Köln, Germany

Parenting behaviors emerge from complex neural circuits conferring sensitivity to infant needs to ensure survival of the species. One important molecular signal for the maternal brain is oxytocin, a nine amino acid peptide produced mainly in the hypothalamus. Oxytocin is believed to powerfully enhance parental behaviors by increasing the salience of sensory cues from the offspring. However, it remains unexplored what sensory cues from infants can activate oxytocin neurons in new mothers. In this talk, I will present recent data showing that hypothalamic oxytocin neurons in maternal mice (dams) can respond to auditory cues from newborns and subsequently release oxytocin. By performing *in vivo* cell-attached and whole-cell recordings from optically-identified oxytocin neurons, as well as fiber photometry of oxytocin cells in awake dams, we found that oxytocin neurons, but not other hypothalamic cells, are activated following playback of pup distress vocalizations to release oxytocin centrally. Using anatomical tracings and functional circuit mapping, we described a neural circuit routing auditory information about infant vocalizations to the hypothalamus. Studies of synaptic plasticity in acute brain slices revealed that persistent activation of oxytocin neurons following pup calls *in vivo* is likely mediated by a long-term depression of synaptic inhibition in these cells. Finally, chemogenetic inhibition of auditory inputs to the hypothalamus, perturbed auditory-driven maternal behavior in dams. Our data show that this noncanonical auditory circuit gates central oxytocin release and maternal behavior in response to infant vocalizations, providing a mechanism for the integration of sensory cues from the offspring in maternal endocrine networks to ensure modulation of brain state for successful parenting

Talks of the parallel sessions

(Sorted by last name of presenting author)

A rhythmic temporal pattern of PTTH signaling and its modulation during pupal-adult transition underlies eclosion gating in *Drosophila*

Emad Amini; Sina Grimm; Ralf Stanewsky; Meet Zandawala; Christian Wegener

Affiliation of presenting author: Julius-Maximilians-Universität of Würzburg (JMU)

Eclosion, the emergence of the adult holometabolous insect from the pupa is a crucial all-or-none behavior directed to the right time of the day by the circadian clock. *Drosophila melanogaster* ecloses in the early morning, governed by the central clock and a peripheral clock in the endocrine prothoracic gland (PG) that produces a key developmental signal, ecdysone. We previously showed that eclosion rhythmicity requires a pair of bilaterally symmetric Prothoracicotrophic hormone (PTTH)-producing neurons (PTTHn) that connects both clocks by PTTH signaling. However, it remained unclear when exactly PTTH signaling is active and how it is modulated to time eclosion. Now, the new chemoconnectomic techniques have allowed us to comprehensively determine the inputs from the central clock and neuromodulatory peptidergic neurons to the PTTHn which express corresponding G-protein coupled receptors. The functional significance of these peptidergic inputs was tested using various eclosion assays. With the help of activity-dependent luciferase reporters, we found that the PTTHn and the PG downstream of PTTH signaling show a circadian activity pattern that is switched on during late pupal development. The results significantly improve our understanding of the mechanism underlying the timing of insect eclosion and the interaction between circadian and developmental timers.

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The mating system of the common house spider *Parasteatoda tepidariorum*

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Mating systems, with varying female mating rates occurring with the same partner (monogamy) or with multiple mates (polyandry), can have far reaching consequences for population viability and the rate of gene flow. Here, we investigate the mating system of the common house spider *Parasteatoda tepidariorum* (Theridiidae), an emerging model for genetic studies, with yet undescribed reproductive behavior. It is hypothesized that spiders belonging to this family have low re-mating rates. We paired females twice with the same male (monogamy) or with different males (polyandry), scored behaviors and mating success and fitness resulting from single- and double-matings, either monogamous or polyandrous. Despite the study being explorative in nature, we predict successful matings to be more frequent during first encounters, to reduce the risk of remaining unmated. For re-mating to be adaptive, we expect higher fitness of double-mated females, and polyandrous females to experience highest mating success and fitness if reproductive gains are achieved by mating with multiple partners. We show that the majority of the females mated once, not necessarily on their first encounter, and the likelihood of re-mating did not differ between monogamous and polyandrous encounters. The number of matings did not affect fitness, indicated by similar offspring production in females. Female twanging of the web, a behavior that likely advertises female receptivity, lead successful matings, suggesting female control. We discuss how the species ecology, with high mating costs for males and potentially limited female receptivity, may shape a mating system with low mating rates.

Genome-wide, non-coding markers challenge current systematics of afrotherian mammals

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Afrotheria are one of the four major clades of placental mammals that have been defined based on molecular data. It unites morphologically diverse lineages with a common ancient origin in Africa: elephants, sea cows, hyraxes, aardvarks, sengis, golden moles and tenrecs. Under the current systematic consensus, afrotherians are divided into an ungulate-like lineage (Paenungulate: elephants, sea cows, hyraxes) and an insect-feeding lineage (Afroinsectiphilia: aardvarks, sengis, golden moles, tenrecs). In addition, the sister group relationships of elephants and sea cows (Tethytheria) as well as of golden moles and tenrecs (Afrosoricida) are well accepted. However, multiple studies using different sets of protein-coding markers resulted in alternative topologies among afrotherian lineages. As protein-coding markers struggle to unambiguously reveal afrotherian relationships, we focused on two different sets of genome-wide, non-coding markers: ultra-conserved elements (UCEs) and afrotherian-specific transposable elements (AfroSINEs/LAs). Both types of markers have been shown to be highly informative to resolve deeply diverging phylogenies and under high levels of incomplete lineage sorting. Using multispecies coalescent tree reconstruction and simulation approaches, our results reveal that there is no clear signal for an ancient split into Paenungulata and Afroinsectiphilia. Although the monophyly of Afroinsectiphilia fits with the most-common topology among gene trees, the vast majority of gene trees does not support it. Moreover, there is equal support for every topology among aardvarks, sengis and afrosoricidans. These results imply that early afrotherians radiated very rapidly. In addition, there is evidence for ancient gene flow among the major afrotherian lineages. Among paenungulates, we found Tethytheria to be paraphyletic and hyraxes as the sister group to elephants. Thus, this long-accepted and morphologically well-supported clade is challenged. Overall, the impact of our results on the understanding of morphological evolution among afrotherians and the assignment of fossils to different lineages needs to be reevaluated.

The millipede genus *Siphonorhinus* Pocock, 1894, with the description of a new species from the southern Western Ghats of Kerala State, India (Diplopoda, Siphonophorida, Siphonophinidae)

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The complex biogeographic history of the Indian subcontinent has resulted in a diverse fauna, with faunal elements from continental Asia, Gondwana as well as Indian endemics. This is also the case for the Indian millipede fauna. Millipedes are slow-moving soil organisms that can only disperse poorly. The Indian millipede fauna consists of over 270 species, including six species of Colobognatha. The Colobognatha consist of four orders, of which only two are known from India: Polyzoniida (1 genus, 3 species) and Siphonophorida (1 genus, 3 species). The order Siphonophorida consists of two families, Siphonophinidae Cook, 1895 and Siphonophoridae Newport, 1844, the latter not yet reported from India. The Siphonophinidae comprises four genera and about 12 described species. Within the four genera of Siphonophinidae, the genus *Siphonorhinus* Pocock, 1894, is the most diverse with eight species, of which three species have been described from the Indian Himalayan region (altitude < 1000 m), based on female specimens. The other five species are known from Southeast Asia and an undescribed one from Madagascar. Here we integratively describe a new species of the genus *Siphonorhinus*, based on a male specimen, using scanning electron microscopy, micro-CT, and COI genetic barcoding. It was hand collected from a midland evergreen forest (altitude 500-600 m) in the southern Western Ghats of Kerala, India. The newly described species differs from the other three Indian *Siphonorhinus* species mainly in somatic characters such as colour, size, number of segments, etc. The male gonopods, typically used as a diagnostic feature in millipedes, are not known from the other Indian *Siphonorhinus* species. However, the gonopods of the new species resemble those of *Siphonorhinus* species described from Southeast Asia. The Himalayan millipede fauna consists mainly of Holarctic faunal elements, while that of the Western Ghats consists mainly of Gondwanan elements. Given the distribution pattern of the genus *Siphonorhinus* and its diversity in the Himalayan part of India, it is possible that this faunal group has an Asian affinity. However, a more comprehensive study with more species and more genetic markers is needed to test this hypothesis. Furthermore, we present the first record of the family Siphonophoridae from India, with possibly two genera and nine species delimited based on COI barcodes. Our results suggest that there is a hidden diversity of millipedes in India.

How to build a leg: regeneration and development in post-embryonic stages of *Scutigera coleoptrata* (Chilopoda)

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Many arthropod species have the ability of regenerating appendages. This feature is often associated with anamorphic development and might sometimes be restricted only to larval or nymphal stages. In most cases, the regeneration occurs progressively, over multiple molting cycles and rarely concludes in a fully regenerated limb. However, some arthropods are capable of so called “explosive regeneration”. This term came into use when the regeneration ability of the house centipede *Scutigera coleoptrata* was documented for the first time in the early 20th century. These centipedes were shown to fully regenerate all 30 legs within one adult molting cycle. When in danger, *S. coleoptrata* adults can quickly and without further injury autotomize their long, slender legs in order to escape. The legs quickly regrow, and they emerge fully developed and ready to use after the next molt. What is more, even their anamorphic larvae, which are developing one to two leg bearing segments per molting cycle, are able to appendotomize their existing legs in the same way and replace them after the next molt. The main objective is to reveal how this escape artist renews its limbs at such an astonishing rate as an adult, as well as in the anamorphic stages. The cornerstone for this is the thorough anatomical analysis of the growing appendages at relevant stages throughout the regeneration process, as well as during the anamorphic development. While this phenomenon differs from most documentations of regeneration in arthropods, our anatomical investigation so far provides a valuable insight into the oddities of this process. Subsequently, a transcriptomic and molecular approach could also be crucial for finding out how regeneration starts and what determines the formation of the limbs, while also shedding more light on our general understanding of arthropod regeneration and development.

Morphology of the raptorial forelegs and strike kinematics of *Hierodula majuscula* (Insecta: Mantodea) in the light of sexual size dimorphism

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Praying mantises (Insecta: Mantodea) show strong morphological and behavioral adaptations towards the lifestyle of an ambush predator. Here, their prominent prey capturing devices – the raptorial forelegs – are employed; a character system that evolved convergently in different groups of insects but is best known and investigated in mantodeans. A sexual size dimorphism (SSD) occurs in almost all species of mantises, resulting in noticeable variations in the appearance of both sexes. Morphology and behavior of mantodeans have been previously investigated, but only a few studies attended to the variations of internal and external leg morphology as well as the kinematics of the predatory strike in the context of SSD. Our investigation focusses on different larval instars of *Hierodula majuscula* (Mantidae), a species showing noticeable SSD in adult stage only. Here, we combine morphological methods (micro-computed tomography and dissection) with kinematic analysis, obtained from high-speed-video analysis combined with tracking approaches, resulting in a comprehensive description of the extrinsic and intrinsic musculature of the raptorial forelegs of both sexes. Our results reveal a similar muscular set-up in all specimens, with no significant differences neither between instars, nor between the sexes. We supplement our results with information on prey capturing kinematics, like: duration, velocity and speed of the different phases of the predatory strike. While the sex has almost no impact on these kinematic parameters of the strike, we found differences between larval stages as well as variations in comparison to previously studied mantodeans.

Variability in ants' perceptions

Melanie Bey; Rebecca Endermann; Christina Raudies; Jonas Steinle; Megha Majoe; Naomi Alex; Lisa Maczkowicz; Volker Nehring

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Many studies focus on ants' ability to learn their own colony odour label. The label shapes a template in the ant's brain that is regularly updated to follow the odour variation the colony goes through. Comparing template and label allows ants to discriminate friends from foes. This is a useful skill to have considering the pressure from parasites and competitors. Ants are not perfect in recognising nestmates and some are better than others. Foragers are generally better than nurses, while for the species with castes, large workers perform better than minors. We hypothesize that experience with non-nestmates or odors can improve recognition, perhaps through changes of the template or establishment of specific non-nestmate templates. In this study, we investigated the variation in ants' perception and how ants adapt their behavior based on their previous experiences with non-nestmate odours. Learning is constant in their tiny brains throughout their lives. Recognition is the perfect example of this.

Spatial Processing in the Hawkmoth Visual System

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Many animals strongly rely on their visual sense, as it provides information about the natural world with particularly high dimensionality. Natural visual scenes contain different spatial frequencies, which can vary across parts of the visual field, while, changing light conditions affect the reliability of the visual signal. This complex sensory input requires a high amount of flexibility in the visual system, hence, it increases the need to filter the visual input. In particular, insects with their limited neural capacities rely on such mechanisms to extract relevant features out of the breadth of information. An important neuropil for such filtering in the insect visual system is the lamina, the first processing stage. Different contrast, luminance, color or temporal properties are transmitted by the lamina's main relay neurons: the lamina monopolar cells (LMCs). Recently, we have shown that one type of LMC performs spatial summation in the hawkmoth *Macroglossum stellatarum*, by integrating information via its lateral processes that reach into neighboring visual processing units, called cartridges. To characterize the potential for spatial integration of the other LMC types, we used serial block-face electron microscopy to obtain a full 3D-scan of one lamina cartridge. Reconstructing the fine structure of all lamina neurons in one cartridge, including their lateral processes, revealed a higher number of LMCs and different morphological characteristics than previously described in Golgi stainings. Based on our findings, we suggest a classification of the LMC types, in coordination with those in other insect groups. Tracing the synaptic connections of the reconstructed LMCs allows us to make predictions about their spatial processing. By combining the physiological properties and anatomical characteristics of the LMCs, we address how the processing of complex spatial input is implemented at the neuronal level.

Functional morphological MR Imaging: a case study on the Antarctic silverfish *Pleuragramma antarctica*

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Modern non-destructive imaging techniques offer distinct advantages for particular anatomical and morphological questions in zoology. The possibility to capture multi-slice images and create 3D representations of complex and fragile morphological structures that can be digitized and animated in combination with image processing techniques are just some of the reasons why these techniques are increasingly being used. Using the Antarctic silverfish *Pleuragramma antarctica* as an example, we show in this presentation how high-field magnetic resonance imaging (MRI) can be used as a tool to address ecological questions in preserved animal samples. *P. antarctica* is a pelagic key species in the food web of high-Antarctic waters and is considered highly vulnerable to changes in its environment. *P. antarctica* is extremely delicate and capturing and keeping individuals of this species alive for *in vivo* imaging studies is hardly possible. Due to the lack of a swim bladder, neutral buoyancy is mainly attained by large amounts of lipids which are stored in lipid sacks. However, the functional role of lipids in *P. antarctica* is not yet fully understood, i.e. it is not clear whether the function of lipids is limited to buoyancy or they serve as energy deposits as well. If the lipids are used as energy storage, differences in the nutritional state should be reflected in the amount of body lipid content. Several examples of imaging applications for addressing ecological questions in *P. antarctica* will be presented. This will include high-resolution morphological 2D- and 3D-MR images from *P. antarctica* to determine body composition and the overall fat and muscle distribution of individual preserved fishes. We calculated the individual lipid and water content from these images, which allowed us to estimate the percentage of overall lipid content. The percentage of lipid content correlated well with literature data obtained from standard, destructive lipid measurement techniques. 3D image reconstructions were used for non-destructive analysis of stomach volume and comparing lipid deposits in different individuals. We show that functional morphological MRI is a useful tool in marine ecological studies, even in preserved samples and provides a suitable alternative to some classical, destructive methods, in particular for studies of fragile and delicate structure.

Analysis of dextran sulfate sodium-induced inflammatory effects in the intestine of the red flour beetle, *Tribolium castaneum*

Anna Christina Böhringer; Lara Deters; Hans Michael Merzendorfer²

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Inflammatory bowel disease (IBD), which includes Crohn's disease and ulcerative colitis, is caused by dysregulation of the mucosal immune system with increased activation of immune cells and release of pro-inflammatory cytokines. This inflammation leads to abdominal pain, diarrhea, weight loss and fatigue. In Germany, more than 300,000 people suffer from IBD and to date only symptomatic treatments exist but no cure. Therefore, further research is needed to develop new and effective therapeutic drugs for IBD patients. Studies to investigate IBD often use mouse models. For this purpose, mice are frequently treated with dextran sulfate sodium (DSS) to induce an inflammatory response, which is very similar to that observed in IBD. The symptoms resulting from DSS-treatment, such as painful diarrhea, intestinal bleedings and rectal prolapse, are highly stressful for the animals. As an invertebrate model for IBD research, we propose the pest model beetle *Tribolium castaneum*. The midgut epithelium of the beetle is covered with the peritrophic matrix, which is a mucous, chitin-containing layer and similar in function to the mucus found in mammalian intestines. Both matrices serve as a protective layer and are sterile at the inner regions close the apical brush border membranes. To analyse the responses to DSS in the intestine, we fed larvae with different concentrations of DSS to elicit inflammatory reactions that possibly mimic those observed in mice. Indeed, we observed a significant growth reduction and developmental delay, but no obvious change in mortality. Optical examination of dissected intestines revealed clear signs of inflammation with multiple local thickenings due to epithelial swelling as revealed by cryosectioning and microscopy. DSS-treatment was found to be associated with an increase in the expression of immune-related genes including TcTOLL, TcDUOX but not TcIMD, all of which have mammalian homologs. We conclude that DSS treatment induces inflammatory responses in the larval gut of *T. castaneum*, which are similar to those observed in DSS challenged mice.

Decoding the basic elements of various chemical communication systems across different insect taxa

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As the earliest and most wide-spread form of communication, chemical signaling has permeated through all known taxa of life. Insects, in particular, have exploited chemical signaling as their primary mode of communication. However, how exactly chemical information is encoded by the vast number of semiochemicals with signaling capabilities remains poorly understood. Cuticular hydrocarbons (CHCs) are an excellent example for this, as they have evolved to convey a vast array of differential chemical information, from sex pheromones to nestmate recognition. CHC profiles constitute complex mixtures of up to hundreds of different compounds, and it has been notoriously difficult to unravel the main signaling components except in a few case studies. My current research aspires to elucidate the underlying mechanisms by which chemical information such as sexual attractiveness, health status, and species-as well as nestmate affiliation can be encoded through differential CHC patterns. Intriguingly, closer chemical investigations of communication modalities as diverse as nestmate recognition in invasive ants, social immunity cues in termites, and sexual signalling in parasitoid wasps, collectively hint at the little investigated potential of methyl-branched alkanes as the main coding compounds. This intuitively makes sense, as this CHC compound class indeed carries the most potential for encoding a wide variety of differential chemical information through the multitude of possible positions and numbers of methyl branches. This suggests that the basic signalling properties of methyl-branched alkanes can be evolutionary conserved across vast phylogenetic boundaries, while the actual communicated information can still vary considerably. Therefore, we hypothesize that this particular compound class contributes the basic elements of a potentially unified, commonly evolved “chemical language” across different insect taxa.

Attachment performance of stick insects (Phasmatodea) on plant leaves with different characteristics

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Herbivorous insects and plants exemplify a longstanding antagonistic coevolution, resulting in the development of a variety of adaptations on both sides. Surfaces with lower adhesion ability for insects evolved on some plants and the attachment systems of some insects adapted accordingly. Stick insects (Phasmatodea) are a group of large herbivorous insects possessing an elaborate attachment system with pretarsal (arolia) and tarsal attachment pads (euplantulae). These insects show a remarkable degree of cryptic camouflage imitating parts of plants witnessing the close association with their habitats. The euplantulae of different species reveal a high diversity of microscopic surface structures, which represent adaptations to the respective environments. While research on the attachment performance of stick insects explored aspects of interactions with various isolated factors under laboratory conditions based on artificial substrates, the attachment performance on natural substrates has not been investigated so far. We examined the attachment ability of two species with different attachment microstructures (AMS) on their euplantulae representing the most common types of AMS within phasmids (smooth and nubby) on selected plant leaves with different surface properties by measuring the pull-off and traction forces of individuals with claws and with amputated claws. The plant species were selected to represent different possible properties of their leaf surfaces (smooth, trichome covered, hydrophilic and superhydrophobic covered with crystalline waxes) and their surface morphology was characterised using cryo scanning electron microscopy and 3D microscopy analysis. The results show that the properties of the leaf surface has a strong influence on the attachment performance of the stick insects. While waxes reduced the attachment forces, trichomes and hydrophilic surfaces in general increased the attachment performance. Strong surface texture was primarily beneficial for interlocking of the claws, due to the fact that the higher attachment forces on these substrates were diminished by removing the claws. The attachment forces on smooth and wax-covered leaf surfaces in contrast were not affected by removing the claws, but influenced the performance of the attachment pads. The attachment forces of the studied phasmid species showed a reduced effect of contaminating wax crystals for the nubby euplantulae compared to the smooth ones.

Soluble adenylyl cyclase coordinates intracellular pH homeostasis in calcifying cells of the sea urchin larva

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Biomineralizing cells concentrate dissolved inorganic carbon (DIC) and remove protons from the site of mineral precipitation. Since mineralization is a dynamic process, pH regulatory capacities and mineralization activity of calcifying cells must be orchestrated with the underlying molecular regulatory mechanisms being poorly understood. Here we report that the acid-base sensing enzyme soluble adenylyl cyclase (sAC) coordinates intracellular pH (pHi) regulation and biominerization in the calcifying primary mesenchyme cells (PMCs) of sea urchin larvae. Single cell transcriptomics, *in situ* hybridization, and immunocytochemistry elucidated the spatial-temporal expression of sAC during skeletogenesis. Live pHi imaging of PMCs revealed that down-regulation of sAC activity with two structurally unrelated small molecules inhibited pH regulatory capacities of PMCs, an effect that was rescued by addition of cell-permeable cAMP. Pharmacological sAC inhibition also significantly reduced normal spicule growth as well as spicule re-growth following decalcification, establishing a link between PMC pHi regulation and biominerization. Finally, increased expression of sAC mRNA levels were detected under skeleton remineralization and during exposure to elevated CO₂ conditions. These findings suggested that transcriptional regulation of sAC activity is required to promote calcification and for compensatory responses to ocean acidification. Overall, these results shed light into the molecular mechanisms underlying cellular coordination of acid-base regulation and biominerization and their responses to environmental disturbances.

How to outcompete a native lizard? Violence is not always the answer.

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At a time when we face a global biodiversity crisis, with a dramatic loss of species worldwide, it is intriguing how some organisms seem to be little affected by changes in their environment. They successfully adjust to different environments (such as invasive species) or to changes that take place in their native range (such as urban dwellers), and understanding how these particular species do this can be of major importance. The Italian wall lizard, *Podarcis siculus*, has several invasive populations in different countries, and is also found living within cities across its native and invasive range. This lizard is thus a great model to investigate an array of questions regarding the success of species that can quickly adjust to changes in their environment. Moreover, it can also impact, displace and even eradicate native species. To test how *P. siculus* can outcompete a native lizard, we conducted a study with an introduced population in Portugal that lives in urban gardens, together with the native Green Iberian wall lizard, *Podarcis virescens*. Remarkably, instead of interference competition, we found exploitative competition occurring between the two species. Both species were tolerant of each other, but the invasive *P. siculus* was first to eat, consumed more food, and gained more weight than the native species over time. Our results indicate the behaviour of both the invasive and native species is likely to be promoting the invasive success of *P. siculus*, and further highlight the importance of investigating behaviour for understanding biological invasions.

Morphological Analysis and Categorization of Ascending Neuron Types from the Somatosensory Layers of the Ventral Nerve Cord to the Brain of Adult *Drosophila melanogaster*

Erica Ehrhardt; Massimo Thiel; Kei Ito

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Deep evolutionary homology is evident in the organization of sensory systems of vertebrates and invertebrates. In both insects and mammals, the axons of primary somatosensory neurons terminate in similar modality-specific layers in the nerve cord. Ascending neurons (ANs) transmit somatosensory information from the nerve cord to the brain, which uses this information to modulate behavior. However, knowledge about the types and compositions of ascending neurons remains limited. In the compact nervous system of insects, single neurons can often be identified and named reliably between different individual animals. In *Drosophila melanogaster*, specific driver lines can be used to visualize and manipulate identified neuron types. The MultiColor FlipOut (MCFO) method produces sparse random labeling of single neurons, enabling the precise visualization of its dendrites and axon terminals. We aligned three dimensional image stacks of single-ANs of approximately 6,000 samples to standard templates. These images were overlaid with the images of previously identified primary somatosensory neurons, to identify the modality of sensory input received by each AN type. We also overlaid the AN images with the images of identified motor neurons, to find the AN types that overlap with motor neuron neurites and thus may encode efference copies. We identified approximately 400 AN types. They can be hierarchically classified into groups of closely associated types based on their morphology, trajectories of their neurites and axons, whether they receive bilateral or unilateral input, the neuropils and sensory layers they innervate in the ventral nerve cord, and the location of their axon terminals in the brain. Ascending neurons that receive input in the leg neuropils of the ventral nerve cord tend to innervate the anterior brain, particularly the Anterior Ventrolateral Protocerebrum, whereas ANs with input in the neck neuropil, dorsal wing neuropil, haltere neuropil or intermediate tectulum tend to target the posterior brain, such as the Posterior Slope or Posterior Lateral Protocerebrum. Additionally, we used our collection of AN typing data with light microscopy to find matching neurons in the electron microscopy connectome data. Identification of AN types and of driver lines targeting them will enable further investigation into the function of somatosensory circuits in insect behaviors such as walking.

The ghost of oysters past - genomic insights into extinct oyster diversity

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Human impact often leads to the decline or loss of native biodiversity. The European oyster (*Ostrea edulis* L.) is a prominent example of such decline. Abundant and cheap seafood in the past, the oyster is today an expensive delicacy. This change is a consequence of the sharp decline of wild oyster populations in the 20th century. In the German and Danish Wadden Sea, the European oyster vanished altogether in the 1950s, and has not been able to reclaim this habitat. We asked: What has caused the extinction and prevents a successful recolonization? Is larval connectivity into the Wadden Sea limited, causing genetic isolation? Was the original population locally-adapted to the specific Wadden Sea conditions, and the required adaptations for a recolonization of this habitat have been lost? Answering these questions requires us to travel back in time to investigate a now-extinct population. The Zoological Museum of the Kiel University holds the key for such time travel: an extensive collection of oyster shells from all over Europe - including the now extinct North Sea population - collected by Karl-August Möbius in the 1870s. Applying museomics to these historical oyster shells, we were able to reconstruct historical phylogeography and conduct genome scans of local adaptation. The mitochondrial genomes show distinct haplotype diversity in the Wadden Sea, suggesting that this population was isolated - but all of this diversity is still present today. Thus, the mitochondrial genomes do not lack the diversity needed to recolonize this area. The nuclear genomes show that the Wadden Sea oysters were even more distinct than the mitochondrial data predicted. This indicates genetic isolation of the Wadden Sea population. The genome scans we carried out provide first evidence of local adaptation: the same genomic regions have elevated F_{ST} values between Wadden Sea and a combined French-British population, and relatively low Tajima's D in the Wadden Sea population. Thus oysters from the Wadden Sea may really have been adapted to life in this harsh environment.

Complexity of scaling relationships indicates an absence of general trade-offs between olfaction and vision in *Drosophila*

Max S. Farnworth; Stephen H. Montgomery

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The diversity in anatomy of nervous systems and sensory organs reflects a complex system of interactions between selection processes, functional integration, developmental constraints, and energy costs. How these elements interact is a long-standing question in evolutionary biology. A recent prominent idea to explain variation in nervous and sensory organs is the concept of trade-offs. A trade-off in such context means that one sensory modality is favoured during evolution and development at the expense of another, such that one sensory organ and corresponding brain area increases in size, while the other decreases. To address this idea, a whole collection of data sets is needed that comprise development, comprehensive anatomical investigation as well as behavioural tests across a set of diverse species. I will present our work that revisited such an impressive dataset to test how pervasive trade-offs are across different modalities. For this, we used MCMC based phylogenetic regression models as well as major axis tests to assess variation in visual and olfactory brain regions, adult eyes and antennae as well as their corresponding developmental structure, the eye-antennal imaginal disc. We find that there is much more variation in the sizes of these structures than could be explained by a trade-off and a corresponding energetic explanation around a shared energy budget. We therefore reach the conclusion that while trade-offs are an intuitive argument to interpret patterns of inverse size relationships in pair-wise species comparisons, it is not sufficient to explain species-wide variation in sensory organ and brain sizes. In our view, vision and olfaction specifically, but other sensory modalities generally, can covary with each other positively, negatively, or not at all across species and most variation cannot be explained by trade-offs.

Neuronal circuits mediating olfactory second-order conditioning in the *Drosophila* brain

El Yazid Rachad; André Fiala

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We use the fruit fly *Drosophila melanogaster* with its sophisticated genetic tools to work towards an understanding of the neuronal mechanisms underlying higher-order associative learning. In *Drosophila*, simple classical conditioning can be achieved by temporally paring a conditioned odor stimulus (CS+) with an unconditioned stimulus (US) that mediates reward or punishment, such as sugar or electric shocks. The neuronal circuitry mediating this type of classical Pavlovian conditioning has been extensively studied and could be localized to the mushroom body of the central brain. However, neuronal circuits mediating the formation of association chains through higher-order conditioning remain unknown. Second-order conditioning can be achieved when a previously conditioned, aversively trained odor stimulus (now referred to as CS1) is temporally paired with a second, novel odor stimulus (CS2), thereby transferring the learned, conditioned response to the CS2. This type of second-order conditioning offers the opportunity to examine how the internal transfer of learned, predictive information from one odor stimulus representation to another one occurs at the cellular and circuit level. By selective, thermo-genetic manipulation of distinct neuronal populations of the mushroom body circuitry during the different phases of first-order training, second-order training or in the memory test situation, we dissected the neuronal connections that are required for aversive associative second-order learning. Calcium imaging was used to visualize synaptic plasticity accompanying the distinct training phases. Thereby, we characterized two mushroom body-associated neuronal feedback loops that enable a learned odor representation to take control over punishment-mediating dopaminergic neurons, thereby mediating associative learning of higher order. The general relevance of this finding will be discussed.

Food quality impacts on life history traits of a freshwater calanoid copepod

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The nutritional quality of phytoplankton is essential for the fitness of herbivorous zooplankton and for efficient carbon fluxes in pelagic ecosystems. In freshwater lakes, cladocerans and calanoid copepods are the main pelagic herbivores in terms of both numbers and grazing impact. However, most studies focused on the easily cultivable cladocerans, while only few studies addressed the impact of the diet on freshwater calanoid copepods due to their more complex life cycle. We here supplied five different phytoplankton diets to the freshwater calanoid copepod *Eudiaptomus* sp. to investigate their dietary quality for the copepods' fitness traits over the copepod's entire life cycle. While all tested diets supported comparable reproductive success in adults, egg production, hatching success and survival rate differed markedly between diets. In the offspring generation, diet affected developmental and reproductive periods, size at first reproduction and clutch size. *Eudiaptomus* body fatty acid composition only partially reflected their diet, indicating that the copepods are able to selectively accumulate and interconvert certain essential fatty acids. This capability may allow them to cope with nutritional deficiencies and may thus be interpreted as an ecological adaptation strategy to the fluctuating environmental conditions and resource availabilities in freshwater plankton.

Do fish prefer live tutoring or e-courses? Do Atlantic mollies make a difference between real and virtual male tutors?

Theodora Fuss; Maya Werthenbach; Klaudia Witte

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Numerous studies have examined social learning and sex differences in many different contexts and with regard to a variety of cognitive tasks. However, what about the social context? Thanks to recent technological developments, computer animations are more frequently used to answer complex behavioral questions. Therefore, it is important to question whether virtual stimuli are in fact as powerful as live conspecifics, especially in the context of social learning and cognitive tasks. In this study, we investigated whether Atlantic mollies (*Poecilia mexicana*) acquire information from e-tutors as well as from live tutors. In a first study, live male Atlantic mollies were taught to find a hidden food reward located in the holes of a hole board covered with colored chips (green, yellow). Upon learning the task, these males served as tutors for other male and female Atlantic mollies. Following a series of tutor trials, an observer was allowed to try to solve the observed task itself. The observers did not have to learn the rewarded chip color only once, but the rewarded color changed randomly during six consecutive tests. This required the observers to pay close attention to both the behavior they observed to acquire a new food source and the color of the rewarded chip to maximize choice accuracy. In a live social context, male mollies showed (a) significantly better performance and (b) significantly higher levels of behavioral flexibility than their female conspecifics. In a second study, we created virtual male Atlantic molly tutors and generated virtual scenes using our "FishSim" software. The virtual tutors performed the same task in the same way as the live tutors before, but virtually on an LCD monitor. Subsequently, the observers were allowed to find hidden food in their test tank themselves as demonstrated by the e-tutor on the LCD screen. Are Atlantic mollies able to learn socially from a virtual tutor to the same extent as from a live conspecific? Are there differences in social learning from live tutors compared to virtual tutors demonstrating a task? Are there gender differences in cognitive flexibility in social learning of virtual tutor fish?

Nutritional regulation of growth in the sea anemone *Nematostella vectensis*

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Most marine invertebrates, including sea anemones, show feeding-dependent growth throughout their life. All major genetic models, however, have decoupled feeding and growth to keep a fixed adult body size. Nutritional regulation of animal growth is therefore poorly understood. To better understand this process, we studied how the sea anemone *Nematostella* responds to feeding and starvation on the organismal, transcriptomic and cellular levels. Juvenile *Nematostella vectensis* show feeding-dependent growth and dramatic starvation-induced shrinkage (by $\pm 90\%$ within 20 weeks). Our transcriptomic analysis uncovered a gradual transition between growth and shrinkage processes: within two days after feeding, the transcriptome is characterized by genes for transcription, translation, and cell division. Beyond five days of starvation, we observed increased transcription of pro-apoptotic and tumor necrosis factor signaling genes. Using flow cytometry and confocal microscopy, we found an increase in S-phase cells and a corresponding decrease in G1-phase cells within 24h post feeding. Only 24h later, the level of S-phase cells drops 3-fold and remains low until re-feeding. Re-feeding leads to a rapid increase in S-phase cells again, suggesting that a significant population of proliferative cells quickly adopts a quiescent state upon starvation and awaits nutritional input to re-enter the cell cycle. During shrinkage, we observed a decrease of median cell size and cell numbers and an increase in apoptosis, which together likely contribute to a reduction in body size. We thus demonstrated in *Nematostella* that feeding-dependent organismal growth and shrinkage are the outcomes of a tight nutritional regulation of the cell cycle, cell death, as well as the genes that underlie these processes. Our findings thus provide exciting opportunities for further investigating the mechanisms and evolution of life-long, feeding-dependent growth mechanisms.

Can plover chicks recognize the smell of their parents?

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Birds can use their sense of smell in a variety of contexts, like foraging, avoiding predators or communicating with conspecifics. In precocial species, chicks can forage on their own directly after hatching, but they still need care from their parents (e.g. thermoregulation). In species with high nesting densities, chicks should solicit care from their parents, and not from unrelated adults, and therefore they should recognise their parents. This parent recognition may be achieved using olfactory cues. To test this hypothesis, we studied white-fronted plovers (*Charadrius marginatus*), a precocial shorebird species breeding in high densities in Madagascar. Using a Y-maze in the field, we tested whether plover chicks could discriminate between the odour of their parents and that of unrelated adults.

Gene expression patterns explain temporal differences in the processes of polyp bailout in *Stylophora pistillata* compared to *Pocillopora acuta*.

Fabian Gösser; Maximilian Schweinsberg; Ralph Tollrian

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Coral reefs worldwide are increasingly facing acute environmental changes. Due to their sessile lifestyle, corals cannot avoid periods of stress by relocating and have therefore developed various strategies to overcome these challenges. One of these strategies, polyp bailout, may allow corals to survive damaging conditions by resettling in a more favorable environment, but is underrepresented in scientific studies. During polyp bailout, the colonial integrity of the coral is broken down, the coenosarc dissolves, and individual or patches of polyps can detach from the calcareous skeleton and be passively dispersed by the water current. In recent years, first insights into signaling cascades of this stress response in *Pocillopora acuta* under different stress regimes were published. Nevertheless, nothing is known about whether these signaling cascades occur in the same pattern in other coral species. Therefore, we performed a stress experiment using coral fragments of *Stylophora pistillata* and, as a control, *P. acuta*, in which increasing salinity levels triggered the polyp bailout response. RNA was extracted from these fragments, followed by differential gene expression analysis by RT-qPCR. Primers were designed for several genes and a reference gene in *S. pistillata*, identified in previous works as being involved in the signaling cascades of various physiological processes during polyp bailout in *P. acuta*. In both coral species, polyp bailout was successfully triggered, and the same genes were differentially expressed. However, there were differences in temporal activity patterns of the physiological processes and the differential gene expression. In *P. acuta*, dissolution of the coenosarc occurred before polyp detachment, whereas in *S. pistillata*, polyp detachment began earlier, before complete dissolution of the coenosarc. This was also reflected in the different temporal expression patterns of the genes studied. The expression levels of detached polyps of both coral species showed normalization of gene expression. Our results show that the same signaling pathways are involved in polyp bailout in different coral species. Further studies will clarify how this stress response unfolds outside of the Pocilloporidae and provide insights whether it is a conserved process within the Scleractinia. This should allow us to better understand the ecological potential of polyp detachment for survival, recovery, and recruitment of stressed coral reefs.

Neither leg, nor jaw, nor all the same: A critical revision of the eumalacostracan maxilliped

Markus Grams; Michael Klinger; Stefan Richter

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Within Malacostraca, legs are variously diversified throughout the groups. Most conspicuous is the transformation of anterior thoracopods, especially the first, into maxillipeds involved in feeding. The concept, however, of what is considered a maxilliped is not very sharp as it relates to a vague combination of morphological and functional deviation from a locomotory limb. While general homology of the first thoracopod (as maxilliped or not) is beyond doubt, special homology of the anteriormost maxilliped remains uncertain. For better insights into this question, we studied the musculature and exoskeletal structures of the first thoracopods in representatives of Anaspidacea, Euphausiacea, Lophogastrida, Mysida and Stygiomysida, using 3D reconstruction of CLSM- and µCT data. Our analysis shows high complexity of muscular and skeletal arrangements in the first thoracopods. In Anaspidacea and Euphausiacea the "maxilliped" differs little from the posterior limbs, though specific correspondences between these taxa might represent synapomorphies. The "mysidacean maxilliped" is morphologically well derived from the posterior thoracopods and appears synapomorphic for the Mysidacea. A comparison with other Peracarida additionally shows correspondences indicating a homologous "peracaridan maxilliped", supporting the monophyly of Peracarida. For a distinction from the decapodan (first) maxilliped, we propose the term "unguiped" for the peracaridan first pair of thoracopods.

Knock-down of vital gene (s) of Tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) using in Planta RNAi

Muneeb Hassan; Faisal Saeed; Haneef Tariq; Ufuk Demirel; Ayhan Gokce; Emre Aksoy; Hans Merzendorfer; Allah Bakhsh

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The South American tomato pinworm, *Tuta absoluta*, is considered worldwide one of the most important tomato pests. Insecticide resistance reported on the moth and the awareness about the negative impact of agrochemicals on the environment and human health have raised the demand for new control tools. Among these, RNA interference is a highly sequence-specific silencing process and can inhibit specific gene functions in targeted insect pests by utilizing dsRNA. RNA interference (RNAi) can represent a valid tool to be included in new control strategies against this pest. Here we report the effects of plant-mediated RNAi targeting acetylcholinesterase 1 (AChE1) and Sec23(COPII). The partial cDNA of AChE1 and Sec23 gene of *Tuta absoluta* was amplified using specific primers in sense and anti-sense orientations and cloned in the pFGC5941 vector with two multiple cloning sites separated by an intron (chsA intron). Cotyledonary Leaf, Cotyledonary Nodes, and Hypocotyls explants of Rio Grande tomato cultivar was infected with Agrobacterium strain EHA105 harboring constructs under the control of CaMV 35S promoter. Standard molecular analysis of primary transformants showed proper integration of T-DNA in the plant genome. The transgenic plants were evaluated for their efficacy against 2nd, 3rd, and 4th instar *Tuta absoluta* larvae. The leaf bioassays revealed 15–60% of *Tuta absoluta* mortality. A significantly lower fold-change in larval weight was observed in insects fed on transgenic plants compared to the ones fed on control plants. Furthermore, *Tuta absoluta* larvae fed on transgenic plants exhibited reduced AchE1 and Sec23 transcripts, indicating the functionality of dsAChE1 and dsSec23 in silencing AchE1 and Sec23 gene expression. Synergism of RNAi with organophosphate conducted on the various instar larvae, exhibited 90% mortality of larvae when fed on leaves of transgenic plants expressing dsAChE1 with reduced dosage application of Hypnose total (Pirimiphos methyl) insecticide. The delivered dsRNA by means of in planta delivery affected the weight of both *T. absoluta* pre-pupae and pupae. The selected AChE1 and Sec23 genes proved to be suitable targets for *T. absoluta* control by means of in planta RNAi. This study is an excellent example of the integration of an alternative, effective and reliable method to cope with tomato insect pests that incur significant losses to Tomato production in the world.

The P5-swimming crab morphotype – once evolved and several times reduced?

Dennis Hazerli; Stefan Richter

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Recent representatives of Portunoidea of true crabs (Brachyura) show great morphological disparity. They are well-known for many representatives showing the – as we call it – P5-swimming crab morphotype, in which the last pereiopods (P5) have the shape of modified swimming legs. However, there are few studies on the evolution of P5-swimming crabs. Thus, it is still not known if the P5-swimming crab morphotype evolved several times independently or just once. Our combined analysis involving molecular and morphological data together with an ancestral character state reconstruction shows that the stem species of Portunoidea already showed the P5-swimming crab morphotype. Thereby, the evolution of some key characters of the morphotype are briefly discussed concerning the „endoskeleton“ and the extrinsic P5 musculature originating at it.

Flying Perfume Makers: On the causes of scent collection behavior in male orchid bees (*Euglossini*)

Jonas Henske; Nicholas Saleh; Thomas Chouvenç; Santiago Ramirez; Thomas Eltz

Affiliation of presenting author: Ruhr Universität Bochum

Perfume making of male orchid bees is one of the most fascinating yet enigmatic behaviors in nature, giving rise to an entire pollination syndrome in the neotropics. Following its discovery in the 1960s, it has continued to draw the attention of researchers, yet its ultimate causes have remained a mystery. While current evidence suggests that perfumes function as sex pheromone analogs, their attraction to females has not yet been demonstrated. Also, the role that chemical information plays in euglossine pre-mating communication remains unknown. Here, we show for the first time that the possession of perfume increases both male mating success and paternity in a captive bee population. We supplemented male *Euglossa dilemma* reared from trap-nests with perfume loads harvested from wild conspecifics. In paired-choice experiments, supplemented males mated with more females, and sired more offspring, than untreated, equal-aged, odorless control males. While perfume supplementation had little effect on the intensity of male courtship display, it changed the dynamics of male-male interactions. Our results demonstrate that male perfumes act as chemical signals during courtship that alter both the behavior of females and male competitors. Both natural and sexual selection have likely shaped the evolution of perfume signaling.

Evolutionary dynamics of the natural *Drosophila* microbiome

Mina Hojat Ansari; Fabian Staubach

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Microbes are important drivers and facilitators of metazoan evolution. Accordingly, studying the role of microbiomes in evolution is receiving more and more attention. Considering that *D. melanogaster* lives in a microbe-rich environment and that its phenotype and fitness are highly dependent on the microbes, it seems reasonable to assume that *D. melanogaster* is under selection to evolve mechanisms to control its associated microbiome for its benefit. In addition, a growing body of evidence points to a heritable genetic basis for microbiome interactions in *Drosophila* and suggests that this basis changes between species and in natural populations. Here, we aim to provide a deeper understanding of how the host genome influences the microbiome and how natural selection might shape microbe interacting loci. Therefore, we performed a microbiome genome-wide association study (mGWAS) to identify loci that interact with the microbiome in a natural, evolutionarily relevant setting.

Speciation in correlation with cave immigration in Tasmanian mountain shrimps (*Anaspides*, Anaspidacea, Malacostraca)

Christoph Höpel; Martin Schwentner; Stefan Richter

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The processes and underlying mechanisms that result in the colonization of extreme habitats such as caves and groundwater, are among the most fascinating topics in evolutionary biology. Special adaptations are necessary for surviving in caves and other subterranean habitats. Permanent darkness and low food availability present major challenges for new colonizers. Studying cave biodiversity allows answering questions about the interaction of adaptation, phylogeography and evolution. The Tasmanian Mountain Shrimps (*Anaspides* spp., Anaspidacea, Malacostraca) offer the unique possibility to study colonization and adaptation to a cave or subterranean habitat in closely related but separate lineages. Within the genus *Anaspides*, colonization occurred many times independently and at different time levels. We studied the genetic differentiation within multiple instances of cave immigration and their influence on the speciation. Genetic differentiation was studied using Sanger Sequencing and ddRAD. One of our main focus areas is the Mt Field National Park, where two closely related species with several lineages can be found. While tarns and pools on the mountain plateau are inhabited by *A. richardsoni*, the mountain range surrounding caves are inhabited by its sister species, the obligate cave dwelling *A. eberhardi*. Our data indicates multiple instances of immigration into the subterranean habitat of the ancestors of what is today *A. eberhardi*, which appear to have occurred during different time periods of the Pleistocene. Interestingly the inflow streams to these caves are inhabited only by distant related species. We found evidence of interbreeding among populations of *A. eberhardi* and the absence of interspecific interbreeding between *A. eberhardi* and *A. richardsoni*. A different situation is encountered in the South-East of Tasmania, where the sister species *A. jarmani* (surface and cave forms) and *A. clarkei* (obligate cave dwelling) occur. Here we have instances of syntopy in some caves and not only do we find shared COI-haplotypes between cave forms of the two species, but also between surface and cave forms. Whether this indicates incomplete lineage sorting or ongoing gene flow is the focus of ongoing research.

Long-term analysis and effectiveness of inducible behavioural and morphological traits of *Daphnia longicephala* in response to *Notonecta*

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The freshwater crustacean *Daphnia* is well-known to adapt to changing predator environments by forming inducible defences. While defensive traits in many species have already been described, the actual mode of action reducing the predation rate is mostly unknown. Furthermore, the ontogenetic time line of defence development is often not described. We investigated inducible behavioural and morphological defences of *Daphnia longicephala* against the heteropteran backswimmer *Notonecta* sp. We documented defensive traits throughout seven successive adult stages. While juvenile *Notonecta*-exposed *D. longicephala* swim slower, *Notonecta*-exposed adults swim faster. We found morphological defences maximally expressed already at maturity. In this stage, we observe a significantly increased body length, an extended crest and an elongated tail spine. During the following adult stages body lengths of both morphs become equal, relative tail spine length is relatively reduced and crest height is kept constant, respectively. Morphological and behavioural parameters were backed up with a second clone. In line with this, we investigated the defensive effect in predation experiments with *Notonecta*. We found that defended *D. longicephala* are more frequently attacked, but also evade attacks more often and thus escape from the predators' grasp more successfully. This significantly decreases prey consumption. Moreover, we tested effectiveness and mode of action of the prey defences. In summary, we provide a comprehensive view on the long-term expression of behavioural and morphological defences in *D. longicephala* in response to continuous exposure to *Notonecta* specific chemical cues.

The proton channel, Otop2L promotes intracellular pH homeostasis critical for skeletogenesis in the sea urchin larva

Marian Hu

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Otopetrins belong to a novel family of proton channels that play a pivotal role in the formation of statoconia and otolith in the vertebrate inner ear with the underlying mechanisms remaining unknown. Using the sea urchin larva, we identified a proton-selective ion channel homolog, Otopetrin (Otop2L), which is exclusively expressed in the calcifying primary mesenchymal cells (PMCs) that generate the larval skeleton. Morpholino knock-down of Otop2L impairs spicule formation associated with changes in the intracellular pH regulatory properties of PMCs. While 70% of PMCs regulate pH_i in a Na⁺-dependent manner 30% of PMCs still recover from an acidosis in the absence of Na⁺. H⁺ currents in these PMCs are sensitive to Zn²⁺ and knock-down of Otop2L reduced proton permeability of the cell. Skeleton regeneration experiments under a wide range of pH indicate a reduction in calcification under acidified conditions underlining the importance of transmembrane proton gradients in the calcification mechanism. Finally, heterologous expression experiments using *Xenopus* oocytes demonstrated that Otop2l displays unique features including strong activation by high extracellular pH (> 8.0) and check-valve like outwardly rectifying H⁺ flux properties making it to a cellular proton extrusion machine adapted to oceanic living condition. Our results provide a first mechanistic understanding how otopetrins contribute to the mineralization process. Protons generated by the calcification process are exported through this proton-selective channel, driven by the negative membrane potential. These findings identified the role of otopetrins in calcifying systems and generate a mechanistic understanding to explain reduced calcification rates in marine species exposed to increased seawater acidity.

Molecular phylogeny of African Hinge-back Tortoises (*Kinixys*)

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The African Hinge-back Tortoise genus *Kinixys* covers eight species which were traditionally assigned to a rainforest group including *Kinixys erosa* and *K. homeana* and a Savannah group including *K. nogueyi*, *K. belliana*, *K. lobatsiana*, *K. zombensis*, *K. spekii*, and *K. natalensis*. However, first studies on the molecular phylogeny of the genus considerably deviated from this traditional concept as well as from each other indicating that phylogenetic relationships and species boundaries within *Kinixys* are not well resolved. In this study I address limitations of previous studies and reinvestigate the molecular phylogeny of the genus using a considerably broader sampling and independent estimates as derived from three mitochondrial and 17 nuclear loci. As exemplified by *Kinixys*, I highlight the vast potential of processing procedures and NGS sequencing techniques specifically designed for fossil and severely degraded historic samples in the face of paucity of fresh material and legislative restrictions hampering research on wide-ranging species.

Phylogenetic distribution of traumatic insemination in Strepsiptera (Insecta)

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Traumatic insemination refers the mating practice of some arthropods in which the male pierces the female's integument with its intromittent organ for insemination. Twisted-winged parasites (Strepsiptera) are considered a prime example of an insect order whose species exercise this mode of copulation. However, while traumatic insemination in the Strepsiptera family Mengenillidae with free-living females is undisputed, its occurrence in Stylopidae with permanent endoparasitic females – comprising the majority of species in this insect order – has remained unclear. Rather, observations from a single study on *Xenos vesparum* (Xenidae) that questioned the role of traumatic mating for insemination in this species were generalized to be representative for all Stylopidae. Here we show that integration of data from various imaging methods provides convincing evidence for traumatic insemination being phylogenetically widespread (including extinct families) and the predominant mode of copulation in the insect order Strepsiptera. Specifically, we provide first-time evidence of injury wounds from traumatic insemination in species of Corioxenidae, Elenchidae, Halictophagidae, and Xenidae. Using micro-CT data to 3D-model the cuticle of copulating pairs of *Stylops ovinae* (Stylopidae) and *X. vesparum*, we additionally visualized the physical piercing of the female's integument by the male's penis in both species. Finally, we show in species of Mengenillidae, Xenidae, and Stylopidae that traumatic mating is associated with the injection of sperm in the female's hemocoel. The results from our study significantly alter our understanding of the reproductive biology of Strepsiptera. They imply that traumatic insemination has been the ancestral mode of copulation in this insect order and retained in most, if not all its extant families.

Effect of Temperature on wide-field motion sensitive neurons in the central brain of bumblebees

Bianca Jaske; Keram Pfeiffer

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Moving animals experience wide-field motion due to the displacement of the retinal image. These cues provide information about rotatory and translational self-motion (Gibson, 1950, Am. J. Psychol.) and allow to estimate different parameters like flight speed (David, 1982, J. Comp. Physiol. A) or distance (Esch and Burns, 1995, Naturwissenschaften), but are also involved in more complex tasks like path integration (Stone et al., 2017, Curr. Biol.). Honeybees and bumblebees are capable of regulating their body temperature above the ambient temperature. Therefore, they use shivering thermogenesis (Stabentheiner et al., 2003, J. Exp. Biol.), so that heat is actively produced by vibrating the flight muscles (Heinrich, 1973, Science). Nevertheless, most studies describe neuronal properties of insect neurons studied at room temperature. Because temperature influences biochemical processes (Montgomery and Macdonald, 1990, Am. J. Physiol. Regul. Integr. Comp. Physiol.) neuronal response properties are affected by temperature. For example, photoreceptors of the blowfly respond faster at higher temperatures (Tatler et al., 2000, J. Comp. Physiol. A). Also, the response of an interneuron of blowflies is faster for higher temperatures and additionally increases in mean spike rate (Warzecha et al., 1999, J. Exp. Biol.). Behaviourally, temperature affects flight properties of bumblebees so that, for example, flight speed and distance is adapted in dependence of environmental temperature (Kenna et al., 2021, Funct Ecol). To understand how temperature might affect tuning properties of wide-field motion sensitive neurons we recorded extracellularly in the central brain of tethered bumblebees while controlling the head temperature of the animal. We compared neuronal responses to moving stripe patterns at different spatial and temporal frequencies for two different temperature conditions: 24 °C and 32 °C. Preliminary data suggest that responses of most units differ in their preferred temporal frequency and mean spike rate. Median preferred temporal frequency increased from 20 Hz to 45 Hz for the warmer condition. Median response gain at the preferred temporal frequency for both temperature conditions was at 1.7. This suggests that motion-sensitive neurons in bumblebees indeed are affected by temperature and are capable of processing wide-field visual motion signals faster for increasing temperatures.

A Gaze into the darkness, shining light into the Moth Flies

Santiago Jaume-Schinkel; GBOL III: Dark Taxa consortium

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The German Barcode of Life (GBOL) III: Dark Taxa is a taxonomy and DNA barcoding initiative funded by the German Federal Ministry of Education, focusing solely on the highly understudied, highly diverse, and unknown parts of insect biodiversity. Illuminating the “Dark Taxa” through modern integrative taxonomy can be achieved by combining morphology, DNA barcodes, genomes, and new researchers, with the ultimate goal of understanding the organisms, the methods, the evolution, and the species limits. After 10 years of the previous phases of GBOL, about half of the animal species occurring in Germany are covered within the DNA Barcode libraries of GBOL, even diverse groups like Coleoptera or Lepidoptera. However, only about 25% of Hymenoptera and 33% of Diptera species are in this GBOL database. These large and megadiverse insect orders are the primary goal of GBOL III: Dark Taxa. One of the targeted groups is the family Psychodidae (Diptera). Commonly known as moth flies, they comprise known vectors of zoonotic diseases, especially human leishmaniasis. Some other species are considered indicators of habitat quality. However, in the majority of species, the biology is unknown. Their tiny size and very often scarce numbers in samples make them a neglected and understudied group among the Diptera. The moth flies are represented in Europe by ca. 500 extant species. Nonetheless, the taxonomic knowledge for moth flies in Europe is uneven, with a few countries having high numbers of studies and records, and many others lack a systematic collection of specimens. This current situation results in many unknown species’ distributions and poor taxonomic treatment for European taxa. Furthermore, the classification of the family and the phylogenetic relationships inside it are poorly understood, hence the necessity of an up-to-date phylogenetic framework that can bring light to the affinities within the group.

Relationship between skull roof bone microanatomy and ecological traits in rodents

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Rodents are the most speciose clade of mammals and show a diverse array of sizes, ecomorphological adaptations, and lifestyles. Despite considerable research on both the external and internal skeletal anatomy of rodents and the adaptive role of different bone structures, no study to date has examined the variability of their skull's inner morphology. The skull roof has the critical role of protecting the brain, provides an area for the attachment of muscles for mastication, and may also play a role in various locomotory activities. Here, we have addressed the question of how the internal bone structure of the rodent skull roof is adapted to their respective lifestyle. We analysed μ CT-scans of 36 skulls belonging to 25 species from ten rodent families. We selected an area from the skull roof bounded posteriorly by the interparietal/parietal suture, anteriorly by the nasofrontal suture and laterally on both sides at the widest point of the frontal bone, and we measured compactness (C), cross-sectional area (CSA), and thickness (T) along the skull roof anteroposterior axis. We then examined the correlation of these parameters with body mass, locomotion (e.g., fossoriality) and diet (e.g., herbivore, omnivore). Mean CSA, mean T and mean C describes the mean value of the respective parameter for the whole selected area. Our preliminary results show that the mean CSA and mean T are both influenced by body mass, so these variables were corrected using skull size as a size proxy. Contrary to our expectations, in our preliminary results, non-fossorial species showed significantly higher mean compactness than fully fossorial species (ANOVA $F=4.648$, $df=2$, $p=0.0167$). Furthermore, primary herbivore species have larger mean CSA (ANOVA $F=7.898$, $df=1$, $p=0.00815$) and mean T (ANOVA $F=7.164$, $df=1$, $p=0.0114$) than omnivores. Whereas a recent study on reptiles found a convergent lifestyle signal in skull roof microanatomy, with fossorial species having more compact skull roofs than non-fossorial taxa, our results in rodents are contrary to these findings. Though preliminary, our first findings show that examining skull roof bone microanatomy of rodents can provide valuable information on the evolution and diversity of this group. Therefore, we have recently expanded our sampling to 118 μ CT-scans of 78 rodent species from 13 families. These will also be examined for adaptations to further different lifestyles (e.g., digging style).

Scalibregmatidae: gain vs. re-gain of palps

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Annelids represent a very old and diverse group within Lophotrochozoa, which can strongly differ in size or shape, and show a huge variety of life styles. Caused by adaptations to different habitats and food sources, in particular the head appendages show an enormous heterogeneity. One very prominent type of head appendage is the annelid palp – a structure with immense morphological variety as well as uncertain homology among different groups. Whereas several annelid families exhibit slender palps for feeding, others show stout sensory palps. In several sedentary annelid families, palp-like structures are lacking at all. A fascinating example in this respect are Scalibregmatidae, where taxa with and without palp-like appendages exist and where recent investigations showed that palp-bearing taxa evolved from “bald” ancestors due to a habitat shift. By using an integrative morphological approach including AZAN-histology, 3D-reconstruction and immunohistochemistry we reconstruct this gripping transformation series from borrowing sister-groups like the Arenicolidae to palp-bearing members of the Scalibregmatidae. Our data enlight the often discussed question whether these palp-like scalibregmatid appendages are homologous to palp-like structures in other annelids or if we observe a convergency in the evolution of head appendages among Annelida. Therefore, our investigations unveil the high plasticity of head structures in Annelida and highlight parallel evolution as a putative key feature driving annelid evolution.

Do Harvester ants use biological control to keep their seeds clean?

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Harvester ants, such as the genus *Pogonomyrmex*, are important seed dispersers in native deserts, prairies and grasslands of arid parts of North and South America. Colony life style involves collecting seeds and storing them in vast quantities for food in subterranean chambers called granaries. While the walls of chambers have been found to be covered in molds and other soil fungi, seeds appear surprisingly clean by visual inspection and are viable to germinate. But, nothing so far is known about the mechanisms these ants use to prevent their stored seeds from spoilage. We here explore the possibility that a seed associated microbiome secretes secondary metabolites that are in effect seed coatings that protect the seed from pathogens. Using NGS methods, we investigate bacterial and fungal microbiomes associated with a harvester ant and their stored seeds. Samples of ants, seeds, chamber and outside soils as negative controls were collected near Tallahassee, Florida, from a *Pogonomyrmex badius* colony. Bacterial and fungal DNA was extracted and 16s rDNA and ITS was Illumina-sequenced to identify bacteria and fungus associated with ants, their seeds and the chamber soils. Preliminary data shows that microbiomes of seeds are dominated by Actinobacteria, a group of bacteria often cultured by other insects as a form of biological control used to protect against pathogenic bacteria and fungi. In contrast, we could not detect Actinobacteria on the ants or in the surrounding soils.

“To Eat or To Be Eaten”: morphological specialisations of predator-prey interactions in the fossil record of insects

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Nearly all organisms exhibit either a predatory or a predator-avoiding lifestyle, sometimes both at the same time. Morphological specialisations to these lifestyles can be grouped according to these: 1) specialisations specific to predatory lifestyles, 2) specialisations specific to predator-avoiding lifestyles, or 3) specialisations that can be found in both aforementioned lifestyles. Morphological specialisations specific to a predatory lifestyle, especially as can be found in the fossil record, can be related to the ability of the predatory organism to better facilitate grasping their prey organism. In insects the most common example of such a structure would be a predatory appendage, as e.g. the predatory forelegs of preying mantises. These predatory appendages are usually characterised by an impressive spination that allow the mantises to better hold onto their prey. Specialisations specific to a predator-avoiding lifestyle, which can be observed in fossilised organisms, can fall into two broad categories: structures for specific predator-avoidance strategies or defensive structures/structures associated with defensive strategies. Morphological specialisations for a predator-avoidance strategy could be specialisations for jumping, as e.g. grasshoppers exhibit a startle reaction by jumping away. Grasshoppers have enlarged hindlegs which they use for jumping. Other defensive strategies can involve spination, enrolment or aggregational behaviour (group formation). Morphological specialisations that can be utilised by both predatory and predator-avoiding organism can be e.g. mimicry, camouflage and masquerade. Camouflage e.g. is used by some predatory insects in combination with a sit-and-wait predatory strategy and as a disguising strategy by predator-avoiding organisms to decrease their predation risk. So there are many ways in which a specific morphology can give insights into potentially exhibited behaviours of extinct organisms by comparison with closely related extant groups and their exhibited behaviour. We show examples of fossil insects in Mesozoic or younger localities that show such specialisations for a predatory or predator-avoiding lifestyle and discuss implications for the evolution of these strategies in different insect groups.

Data from line transect sampling reveal important insights into the bovid community of selected areas of Zambia

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The conservation of bovids is of immense importance, as bovids influence both lower and higher tropical levels, thus playing a vital role in many ecosystems. This includes the Miombo-woodlands, one of the world's most important ecosystems. However, additional research is needed to truly know and adequately protect these areas. In our study, we looked at interactions between population characteristics, habitat use, and behavioral patterns with respect to interspecific and intraspecific differences in the bovid communities of selected areas of Zambia (Kafue Region and Kasanka National Park). We identified interspecific behavioral differences between bovid species of both study sites. Intraspecific analyses were performed on two focus species, the puku (*Kobus vardoni*) and impala (*Aepyceros melampus*). Puku presented various behavioral and ecological variations, for example regarding habitat use in different areas and by different groups, as well as activity patterns in different areas, revealing the plasticity of its ecological niche. Temporal differences in behavior and ecology were also detected. Impala, however, did not show such plasticity. Comparing data from 2009/2010 to 2019, we highlight the increase of flightiness in puku in Kasanka National Park. The data analyzed for different sexes and group compositions provides more than just data-based facts for observations that were previously anecdotal-based (flightiness in territorial males and sunbathing in puku). Moreover, this data provides an important baseline of comparison for future surveys to detect and better understand possible future changes in the populations. Nevertheless, more research is needed to gain a more in-depth insight into the bovid communities of Zambia.

Evolutionary assembly of the neuronal machinery: the role of lineage-specific innovations

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The origin and evolutionary trajectories of neurons remains a matter of debate. It is well established that the first neurons emerged in the last common ancestor of Cnidaria and Bilateria through a step-wise assembly of pre-existing functional modules. Duplication and divergence of pre-metazoan proneuronal genes propelled the duplication and modification, cooption and integration of ancient functional modules such as the presynaptic active zone, synaptic junction scaffold or the postsynapse complex, and hence the birth of the first neurons (Arendt, *Curr Biol* 2020). However, it remains unclear whether the accumulation of these molecular events was sufficient to make up a fully-fledged neuron. The contribution of genomic novelty to the assembly of the neuronal machinery has not been thoroughly investigated so far. Our recent observations on the pre-bilaterian model *Hydra* suggested an essential role of genomic novelty in the molecular makeup of cnidarian nervous systems (Klimovich et al., *PNAS* 2020). Here we trace the evolutionary assembly of the neuronal molecular machinery in the cnidarian lineage using the phylostratigraphic analysis (Domazet-Lošo et al., *Trends Genet* 2007) of *Hydra* single-cell transcriptomic data. We provide evidence for a substantial contribution of lineage-specific genes, particularly restricted to Medusozoa and Hydrozoa, to the neuronal makeup in *Hydra*. Using transgenesis, we demonstrate the role of clade-restricted genes coding for transcription factors, cell cycle regulators and membrane proteins in *Hydra* neurogenesis - the differentiation of neurons from progenitors and neurite outgrowth. Our findings uncover a previously underappreciated role of lineage-specific innovations in the nervous systems evolution. They suggest an independent maturation of the neuronal machinery in the major Metazoa lineages. Our study not only contributes to resolving the evolutionary history of nervous systems, but also provides a paradigm of joint role of conserved genes and genomic innovation in major evolutionary transitions. The work is supported by grants from the German Research Foundation (DFG; CRC 1461: "Neurotronics: Bio-Inspired Information Pathways", CRC1182: "Origin and Function of Metaorganism", project KL 3475/2-1, Croatian Science Foundation project IP-2016-06-5924, and the European Regional Development Fund KK.01.1.1.01.0009 DATAcross).

Transcriptomic response and gill microbial community changes of dominant fish species under steep abiotic gradients in the Elbe estuarine habitat

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Holistic transcriptome studies provide a mechanistic understanding of an organism's response to changing environmental conditions. The connection with physiological and ecological wild fish research allows to elucidate factors for limited resilience and has been increasingly used in the framework of conservation biology in recent years. This study focusses on the linkage of biotic and abiotic factors along the spatio-temporal gradients of tidal Elbe estuary with gene expression patterns in dominant fish species (smelt, ruffe, zander). To account for tissue specificity of transcriptomic adjustments we consider liver as key metabolic organ and gill with respiratory, excretory, and immunological functions. Fish gills require a high connectivity between the surrounding water and the animal's bloodstream, making them an important potential entry point for pathogens. We look at the interaction of the host and its gill microbiome and link this information to various physiological measurements. The aim is to show possible local adaptations of fish of different trophic levels and life history guilds and draw conclusions about factors influencing fish health in the heavily anthropogenically influenced Elbe estuary. For this purpose, fish were caught with a stow-net vessel along the main channel of the tidal Elbe estuary between spring 2021 and summer 2022 and tissue samples and swabs were collected on board. We use holistic transcriptome studies via RNAseq including de novo assemblies along with 16S rRNA gene metabarcoding and LC-MS3 for long-term scale cortisol level determination together with further physiological measurements. First glimpses on the data from summer samplings show clusters in bacterial composition between sites along the estuary but also differences between fish species. Clustering in the microbiome is also reflected in the gill transcriptomes. In the following steps, stress response pathways will be identified from differentially expressed genes and compared between species and seasons. The distribution of potentially pathogenic bacterial taxa and the ratios of bacterial taxa to each other along the estuary and among host species will be identified. Multiomics techniques will be used to show the interplay of bacterial mucus community and the host in response to changing abiotic factors, local adaptations as well as impacts on fish health.

Identification of bioactive compounds from pseudoscorpion venom

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Within Arachnida, scorpions and spiders are well known for utilizing venom to overcome their prey. Apart from these, some pseudoscorpions have also evolved a unique venom delivery system with pedipalps utilized for venom injection. However, the venom composition of this group remains poorly studied. Here, I present our advances in investigating the venom composition of the house pseudoscorpion *Chelifer cancroides*. Accessing the pharmacologic arsenal of these creatures first required the development of a venom extraction methodology which was hampered by the pseudoscorpion's minute size and a unique mechanism preventing the release of venom. Combining electrostimulation with inserting the pseudoscorpion's chelal finger into liquid-filled capillaries finally caused the release of tiny venom amounts. This enabled us to perform the first comprehensive proteo-transcriptomic analysis for a pseudoscorpion. As a result, we identified a large fraction of the dominant venom compounds which comprise potential neurotoxins, antimicrobial peptides (AMP) and enzymes but are dominated by novel compounds with no similarities in the databases. In addition, we performed first activity tests with pseudoscorpion crude venom on a cellular level which revealed a modulation of the insect potassium channel Shaker IR and of voltage-gated sodium channels from the *Varroa* mite. Also, for Checacin1, the first AMP discovered in pseudoscorpion venom, we demonstrated potent inhibitory activities against several bacteria and fungi as well as an insecticidal activity revealed by an aphid feeding assay.

Instinctive fear of snakes?

Sabine Kraus

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Reacting rapidly in threatening situations is crucial for survival. This raises an important question about animals' response to stimuli: when should they learn what is a threat to them, and when should they evolve to innately recognise a threat at first sight? Scattered studies in the literature suggest that genes can encode very detailed and complex visual information that depend on multiple dimensions of appearance such as colour, pattern and shape to form a visual template. Indeed, the need for a rapid innate response to a threat may come with a cost for many false alarms. High specificity of the visual template may therefore be favoured. In a pilot experiment, we investigated the ability of zebra finches to innately recognise a specific, complex visual threat. Zebra finches are a prey species for snakes in Australia. We used wild-type zebra finches derived from first-generation descendants of wild-caught Australian birds. Because they were raised in captivity, they had no previous experience with snakes. We studied and compared the responses of these naïve zebra finches to snake and non-snake stimuli. We measured the latency required for the animals to approach their food or water bath when a stimulus was placed next to it. The stimuli tested were four different snake phenotypes and two control stimuli. We found that the type of stimulus significantly influenced the likelihood that naïve zebra finches would eat or bathe. Specifically, the birds were least likely to eat or bathe in the presence of a small black snake with brown bands or a large uniform black snake. Dark and brown are precisely the predominant colours of Australian snakes. It is not yet clear to what extent the visual pattern of zebra finches is ecologically specific to snakes. Do they generally recognise snake-like shapes, or does their avoidance depend on a specific combination of shape, colour and pattern? However, these initial results are the first step towards further characterising the evolutionary, behavioural, neurological and genetic aspects of the visual templates for snake recognition in birds.

Trophic specialization of Nudibranchia (Mollusca, Gastropoda) reflected by structure, composition and material properties of radular teeth

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Molluscs as the second speciose phylum can be found in almost all habitats, where they established distinct ecological niches. This can also include specialisations to various food items. As molluscs forage with their radula, a thin chitinous membrane with small, embedded teeth, morphological differences (i.e., tooth shape and their arrangement on the radula) are usually associated with specialisations to different food sources. However, mechanical properties as e.g. hardness and elasticity additionally contribute to the radular function and should be included in studies on the evolution of feeding strategies and trophic specialisations. In this context, the nudibranch gastropods are a perfect model group as the taxa show highly specific tooth morphologies and are specialized to distinct prey types. To investigate the functional principles underlying trophic specialisations in nudibranchs, we here focus on seven species, either adapted to feeding on sponges, hydroids, bryozoan, barnacles, or corals. In this study, the mechanical properties of teeth were first tested using nanoindentation technique, which could be directly related to the mechanical properties of the food preferred. Then, the elemental composition of teeth, determined by elemental dispersive X-ray spectroscopy (EDX), was identified to shed light on the origin of these properties. We identified calcium, phosphorus, and fluorine – an indication for the presence of apatite. Additionally, in some species, we detected that the outer chitin layer of the interacting tooth area contained high proportions of silicon, which probably decrease abrasion. Even though radulae showed regional differences in elemental composition, we could not always relate the mechanical properties with the elemental proportions. By applying confocal laser scanning microscopy (CLSM), we however detected strong autofluorescence signals, which finally indicate the relationship between mechanical properties and the degree of tanning.

Facing the Green Threat: A Water Flea's Defenses against a Carnivorous Plant

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Every ecosystem shows multiple levels of species interactions, which are often difficult to isolate and to classify regarding their specific nature. For most of the observed interactions, it comes down to either competition or consumption. The modes of consumption are various and defined by the nature of the consumed organism, e.g., carnivory, herbivory, as well as the extent of the consumption, e.g., grazing, parasitism. While the majority of consumers are animals, carnivorous plants can also pose a threat to arthropods. Water fleas of the family Daphniidae are keystone species in many lentic ecosystems. As most abundant filter feeders, they link the primary production to higher trophic levels. As a response to the high predatory pressures, water fleas have evolved various inducible defenses against animal predators. Here we show the first example, to our knowledge, in *Ceriodaphnia dubia* of such inducible defenses of an animal against a coexisting plant predator, i.e., the carnivorous bladderwort (*Utricularia x neglecta* Lehm, Lentibulariaceae). When the bladderwort is present, *C. dubia* shows changes in morphology, life history and behavior. While the morphological and behavioral adaptations improve *C. dubia*'s survival rate in the presence of this predator, the life-history parameters likely reflect trade-offs for the defense.

First insights into the vocal repertoire of Etruscan shrews

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Vocal communication in animals serves the coordination of social interactions, with different call types emitted in different contexts. An acoustical description of these call types is catalogued in a vocal repertoire. One method to establish a vocal repertoire is the unsupervised cluster analysis, whereby calls are grouped together based on their acoustical properties. Aim of this study is to utilise unsupervised cluster analysis to describe the vocal repertoire of adult Etruscan shrews (*Suncus etruscus*), the smallest extant mammal suggested to be an interesting animal model for hearing research. We conducted 47 social confrontation experiments with 33 Etruscan shrews, whereby two animals at a time were confronting each other. The composition of the dyads differed in sex, familiarity and housing condition. The experiments were video- and audiorecorded. Temporal and spectral acoustic parameters of the vocalisations were measured. Based on these acoustic measurements, a two-level unsupervised cluster analysis was conducted, using both hard and soft clustering approaches, while dimensionality reduction techniques were also utilised. For the behavioural context analysis, a video analysis was carried out. Finally, influencing factors on the call rates were tested with the help of linear mixed models. Four call types ("screech", "screams", "chirp" and "very short tonal") and a transition between the "screech" and the "scream" were described based on the results of the unsupervised cluster analysis. The "chirps" and the "very short tonal" were mainly observed during socio-positive behaviour, while the "screeches", the "screams" and the "screech – scream" transitions were emitted during agonistic interactions. Socio-positive calls occurred mainly in male – female dyads. In addition, an effect of the housing condition on the call rate of socio-negative calls was observed, with the animals housed in same sex groups emitting significantly more socio-negative calls. To conclude, four call types and a transition as well as their behavioural context were described in this study. Nevertheless, additional research is required, since confrontation experiments between unfamiliar animals favoured the occurrence of agonistic interactions. Therefore, recordings in the housing cages are advisable, as they would allow the documentation of a more natural behaviour and possibly additional call types.

DNA barcoding shows evidence for speciation in parthenogenetic and sexual morphospecies of soil-living oribatid mites (Acari, Oribatida)

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Biologists rely on morphological species determination, assuming that species are a coherent entity that can be defined by specific characters. This view, however, is questioned by the application of molecular tools that aid species determination (DNA barcoding) or delimitation (DNA taxonomy). Molecules are independent characters to morphology and have the potential to reveal cryptic species complexes or phenotypic plasticity and even uncovered asexual speciation in bdelloid rotifers. However, asexual speciation is still little explored and the potential of molecular markers to delimit asexual species depends on several factors, such as the origin of asexuality by hybridisation or the age of asexual organisms, which are often evolutionary young. We asked if DNA barcoding can be applied to identify asexual oribatid mite species. Oribatid mites (Acari: Acariformes) are abundant and diverse soil-living microarthropods that significantly contribute to the decomposition of organic matter in soil. Notably, asexual reproduction is common among them and often maintained for millions of years, granting them to be “ancient asexuals”. We analysed the genetic variance of the standard DNA barcoding gene (mitochondrial COI) in two sexual (*Steganacarus magnus* and *Achipteria coleoptrata*) and two asexual species (*Nothrus silvestris* and *Platynothrus peltifer*) sampled across Europe. We tested if morphospecies represent one genetic coherent entity based on the genetic barcoding gap and compared intra- and interspecific variance to congeneric species. The results markedly differed between asexual and sexual species. The asexual morphospecies represented clear genetic clusters with a barcoding gap of ~10-12% that corresponds with other studies on oribatid mites. However, the two sexual morphospecies showed extraordinarily high variation in COI, they split into many genetic lineages and a barcoding gap could not be identified for one species. Our results indicate that genetic variance of COI in asexual oribatid mites is under stronger purifying selection than in sexual species resulting in discrete genetic lineages. These pronounced differences apparently related to the reproductive mode suggest that sexual species keep higher variance in the COI gene, probably to maintain mito-nuclear compatibility between potential mating partners. By contrast, asexual oribatid mites evolve into distinct lineages that eventually may form parthenogenetic species.

Variation in skull morphology in a globally introduced wild carnivore, the small Indian mongoose

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Intra-specific phenotypic variation in wide-spread species can result from environmental gradient, but also from radical changes in ecosystems structure. Such changes can occur when a species is moved outside of its native range. Therefore, invasive species represent interesting models in our understanding of intra-specific variations under changes in environmental conditions and community composition. Here, we investigated the variation in the morphology of the cranium and mandible of a globally introduced wild carnivore, the small Indian mongoose, *Urva auropunctata*. Native to Asia, the species has been introduced into several islands and continental regions during the late 19th and early 20th centuries, most likely from eastern regions of its native range. In order to test for morphological differences at different geographical levels (native versus invasive, between regions and between localities), we used geometric morphometric approaches on a large sample of specimens belonging to several continental native areas and to 21 insular invasive populations. Our results revealed significant differences in size and shape, of both cranium and mandible, between the continental native and the insular invasive populations. Native individuals showed smaller crania and mandibles, and crania appeared thinner and more elongated, suggesting character release in introduced populations. However, when native population was divided into western and eastern parts, only western individuals showed significantly different morphological variations in comparison with introduced populations. Therefore, rather than being related to changes in ecosystem structure, morphological variations in introduced populations may reflect the introduction history of the species.

Generalist-specialist continuum in soil arthropods (Oribatida, Acari): evidence from stable isotope ratios (15N/14N)

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Soil decomposers drive ecosystem functioning through trophic interactions. Many soil decomposers are assumed to feed on a wide range of resources acting as food generalists. However, intraspecific variation in trophic niches of soil animal species is little studied. Here we test trophic specialization using stable isotope ratios of 15N/14N in soil oribatid mites (Acari), the most abundant microarthropods in forests. By focusing on 30 oribatid species each represented by 20 individuals, we found that the variation in trophic position ($\delta^{15}\text{N}$) of oribatid species ranges from one to three trophic levels (from 1.6 to 11 δ unit), indicating that oribatid mites form a continuum of food specialists and generalists. Overall, our findings suggest that the feeding ecology in the populations of soil animal species is heterogeneous, and individuals are not all the same in soil.

Sequence of chondrocranial development in basal anurans

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The craniofacial skeleton is an evolutionary innovation of vertebrates. Due to its complexity and importance to protect the brain and aid in essential functions (e.g., feeding), its development requires a precisely tuned sequence of chondrification and/or ossification events. The comparison of sequential patterns of cartilage formation bears important insights into the evolution of development. The comparison of chondrocranial (cartilaginous neuro- & viscerocranum) development within anurans will help establishing the ancestral pattern of chondrification sequences in anurans and will serve as basis for further studies to reconstruct ancestral conditions in amphibians, tetrapods, and vertebrates. Furthermore, evolutionary patterns in anurans can be studied in the light of adaptations once the ancestral sequence is established. We present a comprehensive overview on the chondrocranial development of different anuran species. With clearing and staining, histology and 3D reconstructions we tracked the chondrification of 73 cartilaginous elements from the first mesenchymal Anlagen to the premetamorphic cartilaginous head skeleton and illustrate the sequential changes of the skull. We identified several traits of anuran cartilage development. In Osteichthyes, neurocranial elements develop in anterior to posterior direction. In the anurans investigated so far the posterior parts of the neurocranium extend anteriorly, while the anterior parts of the neurocranium, extend posteriorly until both parts meet and fuse. Anuran cartilaginous development differs in at least two crucial traits from other gnathostomes which further supports the urgent need for more developmental investigations among this clade to understand the evolution of cartilage development in vertebrates.

Ageing manifests in transcriptomes of brains but not of other tissues in long-lived leaf-cutting ant queens

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Organisms and tissues age at different rates. Mechanistically, life history strategies are thought to trade-off resource allocation between reproduction and body maintenance. This usually manifests itself in organisms that are very fertile having a short life span. In contrast, in social insects such as termites, bees, and ants, the very fertile queens are also the longest-lived insects known, while the genetically similar but infertile workers live much shorter lives. The exact mechanisms of this apparent decoupling of fecundity and longevity in reproductive queens are difficult to analyse, as queens can live for more than a decade and old individuals are harder to come by. We used queens of different ages (up to 16 years old) from established colonies of the Panamanian leaf-cutting ant, *Acromyrmex echinatior* to examine changes in gene expression with age in three tissues (brains, fat bodies, ovaries). Our study is one of the first to thoroughly investigate tissue-specific changes in the molecular regulation of queen ageing. More than 800 genes changed expression with age in the brains of these queens while fewer than 10 genes varied expression in the fat body and ovaries, indicating that most changes that occurred with age were confined to the brain. Brain transcriptional changes indicated senescence in the tissue and mostly involved the conserved insulin/IGF (IIS) and target of rapamycin (TOR) signalling pathways, which regulate many physiological mechanisms, such as nutrient recognition, growth and stress response. For example, we found increased expression of the gene neural lazillo with age, the insect homologue of the mammalian apolipoprotein D, which also increases markedly in ageing human brains. Sirtuin 6 expression, which is typically involved in DNA repair, decreased significantly in the ant brains with age. We did not find any indication that age affected fecundity-associated genes or gene expression in the fat body and ovaries. Indeed, our analysis of ovary development and sperm viability indicated that queens that are more than ten years old should still be highly fertile. Our results suggest that queens may manage to keep up their fecundity for decades by decoupling ageing among tissues that differ in their importance to the queen's role as the germline of the colony.

The hooded seal brain survives hypoxia by decreasing energy expenditure and preventing oxidative damage

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The hooded seal (*Cystophora cristata*) exhibits remarkable diving skills with dives lasting up to 1 hour and reaching depths of over 1000 m. Repetitive breath-hold diving bouts frequently challenge the seal brain with limited oxygen availability while being submerged and oxidative stress caused by reperfusion upon surfacing. While neurons of most terrestrial mammals suffer irreversible damage after only short periods of low tissue oxygen levels (hypoxia), *in vitro* experiments revealed that neurons of the hooded seal show prolonged functional integrity during hypoxic conditions. Little is known that explains the astonishing intrinsic hypoxia tolerance of seal neurons. To advance our knowledge, we stained neurons of the visual cortex of hooded seals and mice and excised them using laser capture microdissection (LCM). The neuronal transcriptomes of both species were compared and we observed reduced expression of genes associated with glutamatergic signaling in seal neurons compared to mice. These results are supported by substrate assays that demonstrated reduced levels of glutamate and glutamine. Released glutamate is a major contributor to neuronal cell death during hypoxia. Therefore, reducing the glutamate pool and glutamatergic signaling possibly saves energy and prevents neurotoxicity. Additionally, antioxidant genes were more strongly expressed in seal neurons, which was confirmed by enzyme assays showing an increased antioxidative activity. Consequently, the “brainy” response of the hooded seal to hypoxia and oxidative stress might include a reduction of energy-intensive processes like neurotransmitter cycling and counteracting the accumulation of deleterious reactive oxygen species (ROS) by an increased antioxidant defense.

Neglected patterns of variation in transgenerational plasticity: the relative importance of maternal, paternal, caring parent and personal risk information varies across ages and sexes in the fathead minnow

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Adaptive transgenerational phenotypic plasticity allows organisms to predict future environmental conditions so as to match their offspring's phenotypes to their environment. Inducible morphological defenses are a well-known adaptive response to elevated predation risk and were one of the first evidence for transgenerational plasticity. However, as research on inducible defenses has traditionally focused on maternal effects, the relative importance of other risk information sources still remains unclear. Additionally, age and sex have been previously highlighted to be major contributors to variation in inducible defenses. Unfortunately, during transgenerational research, inducible defenses are usually only assessed at one point of ontogeny, and results are often pooled across sexes. The lack of comprehensive research contributes to controversial discussions about the evolutionary significance of transgenerational effects. Here, we investigated the inducible defenses of the cyprinid *Pimephales promelas*, which is a sexually reproducing model species with alloparental care. In a first-of-its-kind comprehensive study, we tested the independent and cumulative effects of maternal, paternal, parental care and personal risk environments on inducible defense formation in juveniles, adult males and females. Estimated risk levels were manipulated through continuous exposure to conspecific alarm cues or a water control. With geometric morphometrics, we then studied the body shape of 1555 juveniles and 1367 adults (629 males and 738 females) across 12 risk treatment combinations. Our results reveal age- and sex-specificity in regard to the relative importance of different information sources during morphological transgenerational plasticity.

First results from the joint research project DINA (Diversity of Insects in Nature protected Areas)

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With the project DINA (Diversity of Insects in Nature protected Areas), funded by the German Federal Ministry of Education and Research, the diversity of flying insects and their biomass is recorded nationwide in 21 nature reserves using standardised methods (transect of five Malaise traps at each location). The composition of the insect communities is investigated based on DNA barcoding and additionally – for some taxonomic groups – conventional identification methods. Furthermore, chemical analyses provide information on the pesticide contamination of insects and in soil, vegetation and trees. Chemical analyses from insect-ethanol samples were carried out to screen for the most used agricultural pesticides. In samples from May and August 2020, residues of 47 pesticides were detected, with an average of 16.7 pesticides per study site. Residues of the herbicides metolachlor-S, prosulfocarb, and terbutylazine, as well as the fungicides azoxystrobin and fluopyram were detected at all DINA sites. The neonicotinoid thiacloprid was detected in 16 out of the 21 nature reserves. Statistical analyses clearly showed that the number of detected substances is related to the proportion of arable land within a radius of 2000m around the study sites. Therefore, a reduction of synthetic pesticides in large buffer zones around nature reserves would be needed to minimize contamination of the insect populations. Spatial analyses illustrate the dimensions of arable land in and around nature reserves (NSG) and special areas of conservation (FFH), considering also spatial positional relationships. Based on the German Digital Land Cover Model 2018 (LBM-DE), around 441 km² (NSG) and 1283 km² (FFH) of arable land could be determined. Around 11033 km² of arable land outside these nature protected areas directly adjoin them. These figures provide a valuable contribution to current discussions on the mitigation of ongoing biodiversity loss, as well as current and future necessary amendments of laws and regulations in connection with nature protected areas. Particular designed dialogue formats at three of the 21 DINA sites with the aim to develop recommendations for biodiversity conservation measures will be a substantial contribution to a better understanding of conflicts and aim at establishing joint, robust solutions. This continuous exchange serves both networking between the actors and finding consensus for goals and measures for integrated nature conservation.

Phylogenetic significance of the chaetal arrangement in Annelida

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With approximately 21,000 described species, Annelida represent one of the largest and best studied group of marine invertebrates. More recent transcriptomic and phylogenomic studies yielded an enormous progress in our understanding of their phylogeny that forces to re-evaluate morphological characters. One of their most representative morphological traits are chitinous chaetae, which are present in almost every annelid species. These hair-like extracellular structures are formed within a follicle by a single, specialized epidermal cell, the chaetoblast. The highly dynamic nature of the chaetoblast's microvilli pattern during the chaetogenesis facilitates the formation of numerous different shapes of chaetae. Chaetae often fulfil essential functions for the lifestyle of the respective species, which eminently influences their exact shape. Several chaetal follicles form a chaetal sac, in most cases one per parapodial ramus. Within a chaetal sac, new chaetae are formed at distinct formative sites. The number and positions of these formative sites determine the eventual arrangement pattern of the chaetae. Formation and diversity of chaetae represent relevant characters for identification and description of species as well as phylogenetic and evolutionary analyses. During the past few years, the chaetal arrangement increasingly proved to carry phylogenetic information as well. Data in this regard, however, are still scarce. In order to improve our understanding of the evolution of annelid chaetae, three taxa (Euphrosinidae, Magelonidae and Oweniidae) were investigated with special focus on the arrangement and formative sites of their chaetae. The results indicate that the primary condition of chaetal arrangement in annelids is a transverse row in each parapodial ramus with a single ventral (notopodium) respectively dorsal (neuropodium) formative site. Several alternations of this pattern like staggered rows (Magelonidae), bundles (Oweniidae) or patches (Euphrosinidae) are the result of a shift or multiplication of formative sites or a spiral twist of the row.

Skin development in non-feeding terrestrial tadpoles of *Arthroleptella villiersi* and the evolution of ontogenetic repatterning in frogs with different reproductive modes

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Ontogenetic repatterning is one of the core concepts of evo-devo attempting to explain proximate reasons for the evolution of organismal disparity. It led to the general notion that extensive phenotypic disparity evolved despite general conservation of underlying molecular mechanisms. Modularity offers a solution to this problem allowing the rearrangement of discrete ontogenetic modules via heterochronic, heterotopic or/and heterometric shifts. Frogs represent an excellent model system to identify such ontogenetic modules and investigate the mechanisms underlying their evolutionary transformation. In frogs, aquatic larvae (tadpoles) differ profoundly from their more terrestrial adult forms. The transition from the tadpole to the adult via a climactic, thyroid hormone (TH)-dependent metamorphosis results in the extensive ontogenetic remodelling of almost all organ systems. This plesiomorphic reproductive mode has been altered in many frog species leading to different degrees of terrestrial reproduction. One of the most extreme alterations is a direct developmental mode. Direct-developing frogs lack a free-swimming tadpole and hatch from terrestrial eggs as froglets. In the few species examined, development is characterized by the condensed formation of some tadpole-specific features and the early formation of adult- features during an accelerated, “embryonic”, metamorphosis. Here we compare the skin and thyroidea development between biphasic frogs, the direct-developing frog, *Arthroleptis*, and *Arthroleptella villiersi*. *Arthroleptella* exhibits an intermediate reproductive mode with a terrestrial non-feeding tadpole that metamorphoses into a terrestrial adult. All investigated species exhibit a conserved overall pattern of skin development dividable into an embryonic, larval, metamorphic and adult phase that correlates with histological thyroidea maturation. What differs is the relative timing of skin repatterning. In *Arthroleptis*, the complete skin repatterning takes place during the embryonic phase inside the egg. Additionally, no neuromasts can be detected throughout development. In *Arthroleptella*, terrestrial tadpoles exhibit a larval skin including neuromasts. Neuromast degeneration and skin repatterning to the adult condition is than achieved via a metamorphosis similar to ancestral biphasic species. Our results suggest that the development of adult skin is constrained by the previous establishment of a larval skin pattern and thyroid maturation.

Reconstructing the morphological and chemical evolution of the prothoracic repellent glands in stick and leaf insects (Phasmatodea)

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Phasmatodea are well known for their astonishing ability to imitate twigs, leaves, or bark, and are therefore commonly known as stick and leaf insects. In addition to this primary defensive strategy, many phasmatodean species use prothoracic repellent glands to spray repellent chemicals when disturbed by predators or parasites. We investigate the anatomy and the chemical substances for a representative sampling of stick and leaf insects by use of μ CT scans and gas chromatography/mass spectrometry (GC/MS). The glands are always located pairwise inside the thorax, in some species developed as small sac-like structures, in other species as massive tubes proceeding through the whole thorax. Thus, the size and structure notably differs between species, and several taxa independently evolved huge glands in the New World and Old World Phasmatodea. Regardless of the gland size and structure, numerous stick and leaf insects use the same chemical substance as main compound of their repellent secretion: a bicyclic monoterpenone, which is highly irritating for mucus membranes and the eyes, likewise functions as a repellent against parasites. We could identify the ground pattern molecule of the defensive secretion of the Phasmatodea, which appears to be unchanged in several taxa for over 125 million years. This molecule is often modified, with one or two isomers being used as repellent substance in various Euphasmatodea. Thus, the anatomical diversity of the prothoracic repellent glands is great, whereas the repellent secretion is partly preserved and still present in its ancestral form in various taxa. Still, several taxa lost this ancestral molecule and evolved novel major compounds as repellent secretion, in particular in Necrosciinae and Anisacanthinae.

Birth of neurons and formation of the first neuronal circuit in *Hydra* embryos

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In recent decades, the complexity, function and development of the nervous system aroused attention in the scientific community and focused the point in understanding it's origin and function by observing a variety of different model organisms. The intricacy of various models sharpened the need to find a simple and accessible model to address fundamental evolutionary and developmental questions. Here, we provide initial insights into the development of the first neuronal circuit in *Hydra*, a metazoan of the phylum Cnidaria which belongs to the first animals which contain neurons. As an everlasting embryo, neurogenesis in adult *Hydra* polyps is a permanent process throughout it's lifespan. Differentiation from interstitial stem cells is the key for replacing neurons as an ongoing progress in adult polyps. However, little is known about neurogenesis sensu stricto in *Hydra* embryos. Both the emergence of first neurons as well as the forming of the first neuronal circuit is still largely unknown. Technological advances and new cultivation methods allows the availability of a large number of embryos and here we show that we have identified specific developmental windows in late embryogenesis and post-hatching early hatchlings. Microscopic identifications revealed the absence of mature neuronal cells in embryos at early timepoints. Confocal imaging of neuronal marker proteins supported these data. First findings lead to progenitor cells as a central key to link and form an early nerve net like structure in *Hydra* embryos.

Female fruit flies copy the acceptance, but not the rejection, of a mate

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Preference and avoidance can be socially transmitted, especially in the case of mating preferences. When *Drosophila melanogaster* females observe a conspecific female (called demonstrator female) choosing to mate with one of two males, the former females (called observer female) can memorize and copy the latter female's choice. Traditionally in mate-copying experiments, demonstrations provide two types of information to observer females, namely the acceptance (positive) of one male, while another one is rejected (negative). To disentangle the respective roles of positive and negative information in *Drosophila* mate copying, we performed experiments in which demonstrations provided only one type of information at a time. We found that positive information alone is sufficient to trigger mate copying. Observer females preferred males of phenotype A after watching a female mating with a male of phenotype A in the absence of any other male. Contrastingly, negative information alone (provided by a demonstrator female actively rejecting a male of phenotype B) did not affect future observer females' mating preference. These results suggest that the informative part of demonstrations in *Drosophila* mate-copying experiments lies mainly, if not exclusively, in the positive information provided by the copulation with a given male. We discuss the reasons for such a result and suggest that *Drosophila* females learn to prefer the successful males, implying that the underlying learning mechanisms may be shared with those of appetitive memory in non-social associative learning.

Chemical trait adaptation to new and challenging climates in alpine bumblebees

Sabine Nooten; Johann Neumayer; Thomas Schmitt

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Bumblebees are key pollinators in alpine ecosystems. They are well adapted to these climatically challenging environments, with their short active vegetation periods, high levels of UV radiation, cold temperatures, and dry air. However, bumblebees are declining at an alarming rate, and declines are not uniform. Certain species are more susceptible than others, and this is linked to ecologically relevant functional traits. Here, we investigate the role of desiccation related functional traits in shaping species' abilities to inhabit cold and dry environments. We use complementary field and lab-based approaches to assess the cuticular hydrocarbon (CHC) profiles, body size and age of bumblebee workers. There were marked differences in the desiccation related elements of the CHC profile. Dry environments lead to an increase in the overall quantity of CHCs, while cold and dry environments at high elevations prompted less variation in the chain length of alkenes. In addition, CHC profile responses to climatic conditions were modulated by bee age: older bees had fewer alkenes and longer chains. Our results provide novel insights into trait-based adaptations to dry climates. Given that the frequency and severity of drought events is expected to increase with climate change, we provide a much-needed trait-based understanding of bumblebee declines and show new ways forward for alpine bumblebee conservation.

On springtails (Hexapoda: Collembola): A morphofunctional study of the jumping apparatus

Fabio Oliveira

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The springtails (Hexapoda: Collembola) are tiny organisms that lead a hidden life, mostly occurring deep in the soil and on leaf litter. They have a variety of interesting body morphology patterns, the most famous of which is the catapult-like structure that enables them to jump and flee from predators. This highly specialized jumping apparatus consists of a mobile furca, which when at rest fits into a trigger, "the retinaculum" on the ventral side of the abdomen. Despite the many studies that have attempted to investigate the jumping apparatus, the actual mechanisms involved in the jump, for example the way in which the furca is released by the retinaculum, how and where the mechanisms of spring and hydrostatic pressure originate, are still not properly understood. The morphology of the jumping apparatus of *Orchesella cincta* was investigated in detail using confocal laser scanning microscopy and MicroCT techniques for 3D reconstruction. The abdominal musculature involved in the jumping mechanism and relevant structures of the exoskeleton of retinaculum and furca are described in detail. With the data obtained in this study, hypotheses can be made about 1) where and how the spring and hydrostatic pressure mechanisms originate; 2) which muscles act on the extension and flexion of the furca; 3) which muscles act on the retinaculum and 4) how the retinaculum is released from the furca. The comparative morphological study proved informative, and shows how springtail jumping involves mechanisms unique to this taxon. Hydrostatic pressure regulation possibly varies between animals with distinct segmentation, and those with fused segmentation. Interesting cuticular characters were revealed, such as basal plates and sclerites related to the construction of the spring mechanism. Analysis of videos and images using a high speed camera will be useful for understanding how the jump develops through take-off, aerial and landing phases.

Population analyses with a new automatic photographic recognition software

Pia Oswald; Barbara Caspers

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The global biodiversity crisis affects plant and animal species worldwide, causing severe population declines and extinction events. With 42% species threatened with extinction, amphibians are the most endangered animal taxon affected by habitat fragmentation, climate change and the spread of new pathogens such as the chytrid fungus (*Batrachochytrium salamandrivorans*). Thus, population monitoring is crucial for species conservation to detect possible declines and maintain stable populations. While, in the past, population monitoring was often time-consuming, costly and used invasive markings for individual recognition, recent methods rely on non-invasive marking techniques (e.g., photographs). The use of photographs, however, requires an automated photo recognition software to be time-efficient. We used the newly established "Amphibian and Reptile Wildbook" to perform population analyses of fire salamanders (*Salamandra salamandra*) which have recently been updated to be on the pre-warning list in Germany. In our study population in a forest in Bonn (Kottenforst), female salamanders deposit larvae into ponds and streams. We performed regular larval monitoring and investigated which factors affected the number of captures, mean sizes and individual growth of the larvae from the two distinct habitats. Furthermore, we calculated the estimated population size, survival and recapture rates and tested for the abundance of *B. salamandrivorans* in both habitats. First results indicate no signs of *B. salamandrivorans* and no significant difference between the two habitat types with regard to the number of captures or mean larval size. Our research introduces a new software for individual identification that can be used for non-invasive population monitoring and the observation of individual life histories and provides first insights into population sizes and dynamics of fire salamander larvae in the Kottenforst.

Outsider or mainstream? Larval development of *Palaemon varians* in the Baltic and North Sea

Kira Ovenbeck; Dirk Brandis

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The larvae of most marine decapod species require marine conditions for their development, as they do not yet have significant osmotic regulation. They are only able to tolerate lower salinities after passing through various larval stages. Within the Palaemonidae, however, there are species that have adapted to low salinity habitats (e.g., rivers) by shortening the larval phase so that the development of one or more larval stages is carried out within the eggs. This bypasses the need for marine conditions at the expense of larval dispersal potential, resulting in small-scale dispersal patterns. The brackish water shrimp *Palaemon varians* Laech, 1814 is distributed exclusively in enclosed habitats with sometimes highly variable salinities, such as shallow lagoons or salt marshes. In the Baltic Sea, *P. varians* occurs in areas with very low salinity, which additionally have a highly restricted or no connection to the open sea, raising the question whether the larval development of this species is adapted to these habitats by shortening the larval phase. Regarding this question, we describe for the first time the larval cycle of *P. varians* within the Baltic Sea and compare them with the larval cycle of populations from the North Sea where salinity is significantly higher. Therefore, we use microscopic methods to investigate whether differences in larval development occurred in populations from habitats with extremely different salinity concentrations.

pH and [Na⁺] maintenance in the alkaline midgut of the sea urchin larva: the role of Na⁺/H⁺ exchange mechanisms

Inga Petersen

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Analogous to the situation in several lepidopteran and dipteran insects also the larval stages of some echinoderms and hemichordates (ambulacraria superphylum) evolved highly alkaline midguts up to pH 10.5. Despite its pivotal role in species' sensitivity to changes in seawater pH, the underlying epithelial transport mechanisms are largely unknown. Using ion-selective microelectrodes we found that pluteus larva of the purple sea urchin *Strongylocentrotus purpuratus* not only have highly alkaline midgut fluids (pH ~9) but also a substantial reduced sodium concentration (150 mM) compared to the surrounding sea water (450 mM). We pharmacologically investigated the role of Na⁺/H⁺ exchangers in intracellular pH (pHi) regulation and midgut proton and sodium maintenance using the NHE inhibitor 5 (n ethyl n isopropyl)amiloride (EIPA). While life cell imaging demonstrated the importance of NHEs in pHi regulation of midgut epithelia cells, basolateral EIPA application decreased midgut pH whereas luminal application, via micro-injections, increased midgut [Na⁺], without affecting pH. Gene expression analyses identified putative NHE candidates for midgut pH and Na⁺ homeostasis. Based on this information an antibody was generated against the sea urchin Slc9a2, which showed that the protein is localized in luminal membranes of the midgut. Additionally the use of specific vivo morpholino knock down of spslc9a2 provoked an increase in midgut [Na⁺] without affecting pH underlining the role of this transporter in midgut Na⁺ maintenance. This work provides new insights of NHEs being involved in ion regulatory mechanisms, especially the maintenance of pH and [Na⁺] in larval midgut fluids and thereby show conserved features to insect and vertebrate digestive systems, which may contribute to the ability of sea urchin larvae to cope with changes in seawater pH.

Cryptic diversity in our backyards: The case of a parasitoid wasp

Marie Pollmann; Denise Kuhn; Anna Schmidt; Noa Schwabe; Ronja Reinisch; Irmela Homolka; Sina Paschke; Christian König; Johannes L.M. Steidle

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Hymenoptera contain a large number of described species, but are also known to harbor many cryptic species, especially within parasitoids. This indicates a high speciation rate for this group. Therefore, Hymenoptera are especially suitable to study the process of speciation, and to discover new species, also in unexpected places. *Lariophagus distinguendus* (Hymenoptera: Pteromalidae) (Foerster 1841) is a parasitoid of coleopteran larvae and can be used as a biocontrol agent as many of its hosts are pest on dry stored goods. Recent studies revealed that *L. distinguendus* is a species complex containing at least two species separated by numerous barriers, including different host and habitat preferences, sexual selection, different chromosome numbers, and endosymbiont-induced cytoplasmic incompatibility (CI). To further study the diversity within this complex, we collected additional strains of *L. distinguendus* by exposing traps baited with drugstore beetle larvae in backyards and gardens in the greater area of Stuttgart, Germany. To identify separate species according to the biological species concept, we performed crossing experiments with the collected wasp strains. In addition, the phylogenetic relationship of both established and newly collected strains was analyzed using the barcode segment of COI as well as nuclear markers. Reproductive barriers consisted mostly of sexual isolation and intrinsic postzygotic barriers affecting hybrid males. This agrees with Haldane's rule, stating that intrinsic postzygotic barriers appear first in hybrids of the heterogametic sex. Total isolation between the different strains ranged from close to zero up to 1, i.e. complete isolation. Interestingly, complete isolation was not found in those species pairs which differ by less than 2.9% in COI, but only in species pairs which differ by more than 7.2%. This challenges the common practice of using a 2 % divergence in COI for species delimitation based on molecular data. Our results confirm that *L. distinguendus* is a species complex containing several species, which in turn encompass diverging populations. Remarkably, this diversity occurs in direct proximity to humans, but remained undetected so far.

Can gene regulatory networks help us understanding the evolution of complex traits? What we can learn from natural variation in compound eye size in *Drosophila*.

Ting-Hsuan Lu; Gordon Wieglob; **Nico Posnien**

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Natural variation in complex traits is often controlled by many genomic loci with little individual impact on the phenotypic outcome. Therefore, it is challenging to pinpoint individual genes underlying natural variation in quantitative traits. Since most genes do not act individually but are interconnected in gene regulatory networks (GRNs), the identification of variable nodes and modules within GRNs has a great potential to gain mechanistic insights into phenotypic evolution. The formation of the insect compound eye is determined by a complex GRN composed of more than 5,000 genes and natural variation in eye size is pervasive in *Drosophila*. Therefore, we study differences in compound eye size between *D. melanogaster* and *D. mauritiana* to unravel key variable GRN modules. We integrated quantitative trait loci mapping data with functional genomics information (RNAseq, ATACseq) to reveal 67 candidate genes. An RNAi screen for these genes confirmed a function during eye development for 12 genes. GRN reconstruction allowed us predicting key variable processes underlying observed eye size differences. We confirmed some of these predictions by single-cell RNA sequencing and functional genetics analyses in *D. melanogaster*. We conclude that natural variation in the expression of genes involved in regulating the interplay between cell proliferation and differentiation plays a major role in defining eye size differences among closely related *Drosophila* species. In summary, instead of identifying individual genes underlying eye size variation, we revealed variation in a core network module that is linked to a specific developmental function.

Where is the elusive prothoracic gland equivalent of spiders?

Denise Klinkenbuß; Nikola Michael Prpic-Schäper

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The prothoracic gland in insects is the source of the moulting hormone ecdysone. A similar gland exists in the crustaceans where it is called the Y-organ. Spiders are known to have a similar moulting hormone, ponasterone A, however its source organ(s) in the spider body are not known. The insect prothoracic gland produces ecdysone from dietary cholesterol via a series of enzymatic reactions within the gland. The proteins that perform these enzymatic activities are diverse cytochromes P450, and are encoded by genes collectively referred to as the Halloween genes. We have identified a number of Halloween genes in the spider *Parasteatoda tepidariorum*. We have also identified a [neverland] gene in the spider, the insect homologue of which is also involved in the biosynthesis of the moulting hormone in the prothoracic gland. Since the first moult of the spider embryonic cuticle occurs at the end of embryonic development, we reasoned that the development of the elusive spider organ for moulting hormone synthesis might already develop during embryogenesis and might also express [neverland] and the Halloween genes already before the end of embryonic development. Indeed, we have identified a number of expression domains of the genes under study, especially along the prospective dorsal side of the embryos, all of which represent candidate tissue for the analogue of the insect prothoracic gland primordium, but also appear to be involved in dorsal developmental processes, especially dorsal closure.

Neuropeptidomes and mimetics in Coleoptera

Lapo Ragionieri; Reinhard Predel

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Neuropeptides are among the most ancient and diverse signaling molecules in metazoans, regulating physiological functions such as behaviour, development, and environmental stress responses. Due to their key physiological functions, neuropeptide mimetics have been proposed as candidates for the development of specific insecticides that target key physiological functions of the pests only, without harming beneficial species. Among the Coleoptera, there are many phytophagous species considered as pests worldwide capable of causing serious losses both in cultivated and forest areas. In order to test peptide mimetics as insecticides, we first need to identify lineage-specific features in the neuroendocrine systems of the pests. During the last years information about neuropeptide evolution in Coleoptera increased considerably thanks to the availability of transcriptomic, genomic and mass spectrometric data. Here we summarize recent information available on specifics of peptidergic systems in Coleoptera, with a focus on lineages particularly rich in pest species. For this purpose, we combined transcriptome and genomic data with mass spectrometry analyses to identify neuropeptide precursors and processed neuropeptides from different Coleoptera families; e.g. of *Carabus* (Adephaga: Carabidae) as a natural predator of forest pests, the dung beetle *Anoplotrupes stercorosus* (Polyphaga: Geotrupidae), the forest pest *Hylobius abietis* (Polyphaga: Curculionidae), and *Tenebrio molitor* (Polyphaga: Tenebrionidae) storage pest. At the gene level, the lineages containing many pest species show the loss of several genes commonly found in other insect orders, but no novel peptidergic systems resulting from gene duplications. Therefore, to develop specific mimetics, we need to search within the conserved gene set for derived neuropeptides sequences that are specific only to pests. Such derived sequences were found, for example, in ACP, CAPA-PVK, and PDF. The sequence information obtained is cross-checked with information on other insect orders, such as Hymenoptera, to avoid negative effects of mimetics based on these sequences on beneficial species.

Why such a dramatic decline in puku antelopes *Kobus vardonii* in Kasanka National Park, Zambia? – or why is it so important to know more than the mere numbers of an ungulate population

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There is general consensus that we need to know more about what is going on in our earth's ecosystems. Will still do not know enough about the species themselves, their interactions or about the dynamics. What degree of alternation is normal and when should we be concerned? Monitoring animal populations is a fundamental baseline in conservation. However, data about population size are as important as are data about the population characteristics or distribution. We present here the disturbing example of the puku antelope *Kobus vardonii* in Kasanka National Park, Zambia. This area is considered an important refuge for this more and more threatened antelope where a survey in 2009-2010 estimated a population size of 5,038 (range 3,268–7,238) animals. A re-survey was performed in 2019 that followed the same design in data collection (distance sampling along line transects) and subsequent analyses and thus allowing for direct comparison between survey periods, especially for the data collected in November 2010 and 2019. It revealed a dramatic decrease in population size to only 819 (range 250–2,708) animals in 2019, a decrease of 84%! Data from inside the population (smaller group sizes, decline in male abundance, decline more pronounced at the park boundaries, puku were more vigilant and showed longer flight distances) indicated towards increased poaching to affect negatively the pukus. But the diminished body condition also pointed towards other factors (e.g. below average rainfalls) that contributed to the decline. Other bovid species that were observed in Kasanka National Park in 2010 were observed less frequent or not at all in 2019, making the puku acting as an indicator for the entire bovid community. This worrying outcome shows the immense importance of detailed data that should come along with line transect surveys beyond plain numbers allowing to evaluate changes in populations. In this specific case, it calls for immediate action and an agenda for long-term conservation of Kasanka National Park and its unique ecosystem.

Ants suck – an introduction to the digestive tract of Formicidae

Adrian (Knut Friedrich) Richter; Brendon B. Boudinot; Johan Billen; Evan P Economo; Rolf G. Beutel

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Ants are a dominant factor in almost all terrestrial ecosystems. Their colonies are often considered superorganisms. As in all organisms, the colony must procure and deliver energy and nutrients to its constituent units. An essential organ involved in these tasks is the digestive system (DS) of each individual ant. However, these individuals do not operate in isolation, but rather work as an integrated system to gather, process, store, and distribute food throughout the colony. Ants may derive nourishment from a great diversity of sources: other arthropods, seeds, cultivated fungus, extrafloral nectar, and plant-sucking insect (trophobiont) exudates. The separate colony-units are integrated by different forms of trophallaxis, the exchange of fluids between individuals. Patterns and mechanisms of food sharing are thought to underpin taxon-specific colony-level social attributes. In many species, trophallaxis also serves communication by sharing socially relevant secretions. This implies that the DS is also a means to facilitate and regulate the spread of information in the ant society in addition to energy. In the past, anatomical work on the digestive system has to a large degree focused on the proventriculus. Only recently, our research has uncovered interesting differences in the architecture of the cephalic sucking pump, which may be relevant to the behavioral variation in material uptake and sharing in ants. These differences mainly concern the sclerotized parts of the prepharynx, the sucking pump musculature, a complex of glands, and also the maxillolabial complex, which interacts with the labrum. In my talk I will summarize published and unpublished results regarding variability of the formicid cephalic food processing system as well as the digestive tract in general. I will discuss potential phylogenetic implications and functional relevance and propose possible avenues to deepen our currently highly limited understanding of these questions.

Evolution of incipient toepads and claw morphology in a radiation of ecologically diverse geckos

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Claws are a universal trait among amniote vertebrates that often promote attachment to inclined surfaces in climbing species. Adhesive toepads have evolved repeatedly in a variety of clades for the same purpose. The interaction of the evolutionary processes driving each adaptation, and the potential interaction between them, are still not well understood and recently have received increased scientific attention. Among amniotes, geckos are the most thoroughly studied with regard to their adhesive toepads. Even so, the evolutionary origin of these complex structures is still elusive, this being partially due to the scarcity of studies on species with incipiently developed toepad morphologies. One suggested model for the presence of incipient toepads is the bent-toed Geckos (Genus *Cyrtodactylus*), an ecologically diverse radiation, the climbing members of which possess enlarged subdigital scales. It is known that a few such species carry spatulated setae on these scales. Given this, we explore the evolution of claw and incipient toepad morphology in *Cyrtodactylus* in relation to habitat use to unravel I) if both traits evolve in parallel or independently of each other and II) if any interaction revealed is correlated with the habitat in which locomotion takes place. We measured, relative to body size, subdigital scale area (as a proxy for incipient toepad development) and claw parameters (length, height and curvature) of *Cyrtodactylus* species from different microhabitats (terrestrial, arboreal, rock-dwelling and generalists) to reconstruct the evolution of these traits and to test whether species occupying different microhabitats have differentially evolved either their claws or their subdigital scales or both, repeatedly. We found that as a general trend, both arboreal and rock-dwelling species had relatively larger subdigital scale areas and more strongly curved claws, indicating correlated evolution of toepad and claw morphology, although the degree of morphological changes differed between both traits and among subclades as well as with fine scale microhabitat use (e.g., trunk-dweller vs. crown-dwellers or granite vs. karts formations).

Variation in winter energy use is determined by altering a critical transition period in diapausing *Pieris napi*

Kevin T. Roberts; Philipp Lehmann

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Long winters in seasonal environments present massive energetic challenges for organisms inhabiting them. To survive winter, it is critical to manage resources, by both conserving stores, and by synchronizing timing to maximize available resources in spring. The level of energetic suppression and timing of termination of dormancy is highly dependent on local winter conditions, which leads to local adaptation in winter phenotypes. Insects regulate winter energy use by entering diapause, a programmed dormancy, associated with halted development and suppressed metabolic rate. Diapause generally terminates mid-winter and is followed by a quiescent state allowing insects to exit dormancy if conditions become favorable. Locally adapted populations can fine tune energetic savings by altering either the extent of energy use in winter or by altering the timing of the transition to post-diapause quiescence. We currently do not have a good understanding of how locally adapted populations alter these processes to determine energetic savings in winter. Here, we measured metabolic rate-temperature relationships and diapause termination timing of four populations of the butterfly *Pieris napi* from across a wide latitudinal cline spanning Sweden. We found that metabolic rate-temperature relationships do not differ between populations during diapause, but they do increase after the transition from diapause to quiescence. We also found that diapause termination rate is delayed in northern populations that experience colder winters. Together this suggests that local adaptation of energy regulation in diapause is likely achieved through the modification of the timing of regulatory transition periods and not through modification of the thermal sensitivity of underlying metabolic mechanisms.

Metabolic Adaptation and Physiological Resilience in Animals

Nicolas Rohner

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Adapting to extreme environments requires drastic changes to an animal's metabolism. Adaptation to the total darkness and food limitation of caves can be particular challenging. The cavefish *Astyanax mexicanus* is a promising research organism to unravel the genetic basis of starvation resilience. Extant surface and cave morphs of the same species remain interfertile and can be bred outside their natural environments. We have previously shown that cavefish evolved impressive adaptations such as increased appetite, starvation resistance, and altered feeding due to mutations in mc4r. In addition, we found that cavefish display elevated blood sugar levels and insulin resistance caused by a mutation in the insulin receptor. In contrast to human patients, carrying the exact same mutation, cavefish do not display common markers of diabetes and live long and healthy lives. Furthermore, cavefish develop hypertrophic visceral adipocytes without obvious signs of inflammation due to reduced amounts of pro-inflammatory cytokines. Taken together, our work suggests that cavefish develop these phenotypes as part of their starvation resistance and have evolved resilience phenotypes that allow them to tolerate stark deviations from what would be considered normal physiology in other vertebrates, including humans. Recently we have started uncovering the regulatory networks underlying these and other phenotypes of these fish using state-of-the-art technologies. This positions cavefish as a promising model to gain mechanistic insights into disease phenotypes from an evolutionary and adaptive perspective.

Dynamic properties of compass neurons in the bumblebee brain

Lisa Rother; Anna Stöckl; Keram Pfeiffer

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Many insects use the polarization pattern of the sky for spatial orientation. Polarization information is processed in the sky-compass pathway and provides a reference for compass neurons in the central complex (CX). Flying bumblebees typically perform rapid saccadic yaw turns, at angular velocities of up to 2000°/s, alternating with translational flight (Boedekker et al. 2015), which creates a highly dynamic input into these neurons. Most electrophysiological experiments that investigated the tuning of CX-neurons with respect to the angle of polarization (AoP), however, have used stimuli that rotate at a slow and constant velocity, and are lacking these dynamics. Here we recorded intracellularly from compass neurons in the CX of bumblebees, using naturalistic stimulation with polarized light. To simulate the stimulus dynamics a bumblebee experiences during flight, we stimulated using a linear polarizer, that was backlit by a UV LED (365 nm), and rotated according to head orientations obtained from freely flying bumblebees (Boedekker et al. 2015). We found consistent spiking patterns of CX-neurons during repeated presentation of the naturalistic stimulus, showing that these neurons reliably encoded the stimulus. We also noticed that identical AoPs elicited different levels of activity, depending on stimulus history. Peak activity often occurred after periods of inhibition, while total inhibition often occurred after periods of strong excitation. To better understand the responses to the naturalistic stimuli, we designed a rate-code model of neuronal activity that implemented the neuronal response to the AoP as a cos²-function and included the observed effects of spiking history by subtracting a percentage of the average activity in a rectangular window preceding the current stimulus. Fitting the model to our recorded neuronal responses showed that including spiking history activity in the model always improved the fit. Because the firing rates of the neurons depend both on the current AoP, as well as on the previous activity level of the neurons, it is not possible to extract AoPs from the firing rate of an individual neuron. We therefore extended our model to a population of neurons with identical properties, except that every neuron had a different preferred AoP. We show that this population can encode the correct AoP and that the spiking history in the system allows to faster signal a new heading direction after a turn.

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Macroevolutionary patterns of bite performance in insects

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In vertebrates, the macroevolution of maximum bite force is usually phylogenetically structured and often correlated to ecological demands of the animals, such as food preference or competition. In insects, on the other hand, bite force data is extremely scarce and it remained unknown if similar patterns of bite force evolution occur in this megadiverse clade. Here, we present an analysis of *in vivo* bite force measurements of more than 650 biting-chewing insect species across 111 families and 13 orders. We recorded both maximum force values and bite curve shapes (force over time) of over 3,000 individual bites and found an isometric relationship between maximum bite force and head volume (both log₁₀-transformed: $R^2 = 0.69$; $p < 0.001$). Significant predictors of size-corrected maximum bite forces in insects are feeding mode, general head phenotype, and flight ability. However, when taking phylogeny into account, only flight ability remains as a significant but weak predictor of maximum bite force across insects. The analysis of bite curve shapes revealed well-defined differences between hemimetabolous and holometabolous orders: Hemimetabolous insects (Palaeoptera and Polyneoptera) generally exhibit sinusoidal bite shapes, while holometabolous insects show bites with plateau-shaped peaks. This difference in bite curve shapes between hemi- and holometabolous insects apparently evolved at the split between these groups ~345 mya, but the reason for this and why it was so strongly conserved remains yet unclear.

Lipogenesis in parasitic wasps: a fitness relevant rather than a lost trait

Joachim Ruther

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Fatty acids and derivatives thereof are of crucial importance for virtually any organism on earth. Therefore, the ability to convert carbohydrates into fatty acids, a process commonly referred to as lipogenesis, is thought to be a highly conserved trait shared by organisms from a wide range of taxa. This view has been challenged in the past by studies reporting that newly emerged individuals of many parasitic wasp species did not accumulate additional lipid mass despite having had unlimited access to carbohydrates. The missing lipid accumulation in many parasitic wasps has been often equated with their general inability to synthesize fatty acid derivatives de novo from sugars and has been interpreted as an evolutionary metabolic trait loss. Contrary to this hypothesis, we have demonstrated de novo biosynthesis of fatty acids from ^{13}C -labelled α -D-glucose in seventeen species of parasitic wasps from seven families (Prager et al., 2019, Insect Biochem. Mol. Biol; Ruther et al., 2021, Proc. R. Soc. B). This suggests that the ability of parasitic wasps to convert carbohydrates into fatty acid derivatives is a common feature in parasitic wasps. We used the model organism *Nasonia vitripennis*, one of the species that was previously thought to lack lipogenesis, to study the biological significance of de novo fatty acid biosynthesis in parasitic wasps in more detail (Multerer et al., 2022, Proc. R. Soc. B). Using a ^{13}C -labeling approach, we found that glucose-fed females synthesized palmitic, stearic, oleic and linoleic acid as well as di- and triacylglycerides de novo even when their lipid reserves were still intact. Lipogenesis rates, however, increased drastically at higher concentration of the offered sugar source and in females with partially depleted constitutive fat reserves due to previous egg laying. We furthermore found that sugar-fed females incorporate de novo synthesized fatty acids into their eggs and lay more eggs than water-fed control females. We conclude that lipogenesis is a fitness-relevant trait in parasitic wasps and that glucose feeding impacts the fatty acid status not only by decelerating the catabolism of constitutive fat reserves but also by partially replenishing ebbing fat reserves by de novo biosynthesis.

Loud calls of male vervet monkeys as vocal displays of male quality

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Male vervet monkeys *Chlorocebus pygerythrus* produce distinct loud calls in response to their main land predators and occasionally during within and between group conflicts. These ‘alarm barks’ deter predators and alert group members to the presence of potential threats, which decreases predation risk for signalers, their offspring and potential mating partners. Since adult males constantly compete for rank and occasionally produce barks during agonistic interactions, it has been suggested that these loud calls also function as indicators of male quality. Inter-individual variation in calling frequency could provide receivers with cues regarding the motivation, stamina and potential competitive ability of the signaler. Here, we investigate whether male rank, group composition and the mating season predict inter-individual differences in loud call usage. From April 2020 to April 2022 we collected data from 45 adult males living in six groups at the Mawana Game Reserve, KwaZulu-Natal, South Africa. We recorded data on individual male participation during naturally occurring loud call events in addition to individual audio recordings. We hypothesized that male loud calling activity is under intra-sexual selection and expected a positive relationship between individual rank and the probability to produce loud calls during calling events. We further expected a relative increase in individual calling probability during the mating season and in relation to the adult sex ratio and the number of adult males in the group, as these factors contribute to the degree of male-male competition. Our results suggest considerable inter-individual differences in male calling activity, with high-ranking males showing increased participation rates compared to lower ranking ones. These results corroborate the idea that male loud calls are under sexual selection, and fulfill the dual function of alarm calls and indicators of male quality.

Sex specific vocal behaviour in white rhinoceros and its implication for mating

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Vocal communication plays an important role for the coordination of mating behaviour in animals. Vocal exchanges between sexes can give important insights into their social relationship and can indicate the reproductive phase of females. In this project, we investigated sex-specific vocal behaviour in the captive white rhinoceros *Ceratotherium simum simum* by correlating vocal and social behaviour with hormonal data. Since males and female live separately, finding a mating partner at the right time is important for species survival. We conducted audio and video recordings of 7 males and 27 females from 10 zoological institutions (~10 hours per subject over a period of 1 to 2 months). Additionally, we collected faecal samples to analyse the level of testosterone metabolites in males and of oestrogen and progesterone metabolites in females. We obtained the call rate of the four most common call types Snort, Hiss, Grunt and Pant. Conducting a video analysis, we obtained the rate of affiliative, aggressive, defensive social interactions between group members as well as the amount of time the group members spent together. We compared these data between the receptive and non-receptive periods of a female to investigate signals of mating proceptivity. Using vocal network analysis, we found sex-specific differences in the vocal behaviour of white rhinoceroses. Females emitted more aggressive calls than they received and directed aggressive calls more often to males than to other females. Males emitted more affiliative calls than they received suggesting an active role in attracting females. Comparing vocal and social behaviour during the receptive and non-receptive period of female rhinoceroses indicated sex-specific signalling of mating proceptivity. Males increased the rate of affiliative and agonistic calls, whereas females increased presenting and marking behaviour during the receptive compared to the non-receptive periods. Overall, we found sex-specific vocal behaviour indicating a sexual power asymmetry in the white rhinoceros. We suggest that females can control mating by rejecting the male or leaving the territory, whereas males have to play an active role to keep females in their territory by increasing affiliative behaviour. Moreover, male vocalisations and female marking behaviour can serve as a real-time indicator for the receptive period of a female having the potential to improve reproductive management strategies in captive white rhinoceroses.

The slow evolving genome of the xenacoelomorph worm *Xenoturbella bocki*

Philipp Schiffer; Peter Sarkies; Maximilian Telford

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The evolutionary origins of Bilateria remain enigmatic. One of the more enduring proposals highlights similarities between a cnidarian-like planula larva and simple acoel-like flatworms. This idea is based in part on the view of the Xenacoelomorpha as an outgroup to all other bilaterians which are themselves designated the Nephrozoa (protostomes and deuterostomes). Genome data, which can help to elucidate phylogenetic relationships and provide important comparative data, remain sparse for early branching bilaterians. Here we assemble and analyse the genome of the simple, marine xenacoelomorph *Xenoturbella bocki*, a key species for our understanding of early bilaterian and deuterostome evolution. Our highly contiguous genome assembly of *X. bocki* has a size of ~110 Mbp in 18 chromosome like scaffolds, with repeat content, and intron, exon and intergenic space comparable to other bilaterian invertebrates. We find *X. bocki* to have a similar number of genes to other bilaterians and to have retained ancestral metazoan synteny. Key bilaterian signalling pathways are also largely complete and most bilaterian miRNAs are present. We conclude that *X. bocki* has a complex genome typical of bilaterians, in contrast to the apparent simplicity of its body plan. Overall, our data do not provide evidence supporting the idea that Xenacoelomorpha are a primitively simple outgroup to other bilaterians and gene presence/absence data support a relationship with Ambulacraria.

Innovation by inflammation? – The formation of a novel egg-anchoring structure in pelvic brooding ricefishes

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Across the animal kingdom many species increase the fitness of their offspring by providing some form of parental care. External brooding fish carry their developing eggs attached to their bodies, which requires the innovation of novel morphological attachment structures. Pelvic brooding ricefishes evolved such a novel tissue; the so-called “plug”. After spawning, the developing plug anchors egg-attaching filaments inside the gonoduct and thus enables the female to carry the eggs until hatching. Pelvic brooding is described for four ricefish species (*Adrianichthys oophorus*, *Oryzias sarasinorum*, *O. kalimpaaensis*, and *O. eversi*) endemic to freshwaters in Sulawesi, Indonesia. However, the formation and degeneration of the plug is only briefly described for *O. sarasinorum* where the plug is formed by attaching filaments, epithelial cells, blood capillaries and collagen fibrils. In the present study, we sampled females of *O. eversi* at several brooding and non-brooding stages and produced histological sections and μ -CT-scans. To comparatively study the formation of the plug we further sampled females of *A. oophorus* and *O. sarasinorum*. We show that in *O. eversi* the plug is formed by several types of interstitial cells, blood capillaries, and collagen fibrils that encapsulate the end of the attaching filaments in the anterior part of the gonoduct. The additional presence of multinucleated giant cells (fused macrophages) indicates that the ricefish plug evolved due to an inflammatory reaction. We assume that it forms as a reaction to irritation or injury of the gonoduct epithelium by the attaching filaments, similar to a foreign body granuloma. Even 15 days after losing the protruding attaching filaments, the plug remains present. In contrast, in *A. oophorus* the entire plug was absent and instead the gonoduct surrounding sphincter-like structure seemed to be modified. This is in line with phylogenetic evidence indicating that pelvic brooding evolved independently from ancestral transfer brooding in the two distantly related lineages *Oryzias* and *Adrianichthys*. The study concludes that the plug within *Oryzias* may be an evolutionary innovation induced by a modified inflammatory reaction.

No direction home: how ants perform systematic searches

Patrick Schultheiss

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Navigation is of crucial importance for the survival of many animal species. To guarantee the successful completion of a journey, insects engage in highly efficient systematic searches to finally pinpoint their target. The resulting search patterns are highly structured – systematic – while retaining strong adaptive flexibility. Yet, it remains unclear how this sophisticated behaviour is generated by the insect central nervous system. Our current knowledge suggests that it involves the integration of several distinct navigational steering mechanisms in the insect brain. Here, I investigate the extent to which previous visual experience guides ants during their systematic searches for the nest entrance. When experimentally restricting the area around the nest to prevent foraging ants from forming visual memories, I found that subsequent nest searches retained their systematic structure but suffered from very low precision. This indicates that searching ants employ visually guided steering mechanisms, in which the perceived visual environment is matched to visual memories. When such memories are lacking, the steering mechanism is impeded. Further investigations explore the contributions of compass-guided, as well as innate, steering mechanisms to the systematic search. Finally, chemical lesions of targeted brain tissues can provide insights into the neural architecture that generates systematic searches. As a whole, these findings reveal whether highly sophisticated systematic searching behaviour is indeed generated by the interplay of innate movement routines and navigational modules, and how these routines interact with external cues. More broadly, the outcomes allow us to ask questions of fundamental importance about how brains work to produce adaptive behaviour.

Towards a mechanistic understanding of caste polyphenism in social insects

Eva Schultner; Jan Oettler

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Division of labor between reproductive queen and non-reproductive worker castes forms the basis of superorganismality, thereby permitting one of the major transitions in evolution. A century of research on caste determination and differentiation in social insects has revealed a complex picture of how these developmental processes are regulated across the range of queen-worker polyphenism. Today, advances in molecular methods are paving the way toward the identification of common patterns across species, bringing us closer to a unified concept of caste development. I will give an overview of the current state of knowledge on caste polyphenism in social Hymenoptera and present new results on the regulation of embryonic caste development in *Cardiocondyla obscurior*, an ant with an obligately sterile worker caste.

The contribution of hybridization and inflammation on the evolution of a derived reproductive strategy in fishes

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Studying the underlying genetic causes of complex phenotypic traits requires a system in which different characteristics of traits occur in closely related species, which can – ideally – still interbreed. In this respect, ricefishes are an ideal model. Lineages of ricefishes (Beloniformes: Adrianichthyidae) on Sulawesi evolved an extraordinary reproductive system, called “pelvic-brooding”. In contrast to most other ricefishes that deposit fertilized eggs shortly after spawning, females of pelvic-brooding species carry an egg-clutch until the fry hatches. The eggs are connected with each other and the female by attaching filaments and anchored inside the female’s abdomen by a unique structure, called plug. Ovulation is suppressed during egg-carrying resembling pregnancy. To tackle the phenotypic and genomic bases as well as the evolution and adaptive value of this brooding strategy we use a holistic approach linking comparative morphology, genomic investigations and field data. Here, we present latest results on the genomic architecture of pelvic brooding, the role of inter-specific hybridization and the contribution of a modified inflammatory reaction to the evolution of a novel tissue related to this derived brooding strategy.

Discovery of a cryptic species among samples of one of the most common European vespid wasps, *Polistes dominula* (Hymenoptera: Vespidae)

Fabian Schweitzer; Leander Bertsch; Luca Mehlhorn; Oliver Niehuis

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The vespid wasp *Polistes dominula* (Christ, 1791) is one of the most common paper wasps in Europe and considered an invasive species in all temperate regions of the world outside of their indigenous range. Previous investigations revealed that its mitochondrial (mt) genomes belong to two distinct haplotype groups that are phylogenetically paraphyletic in respect of *Polistes bucharensis* Erichson, 1849. Whether wasps with mitochondria from the two haplotype groups are reproductively isolated from each other has remained unknown, however. Using a Pool-Seq approach for genome-wide screening of single nucleotide polymorphisms in combination with comparative analyses of cuticular hydrocarbon (CHC) profiles and of wing landmarks, we show that *P. dominula* females with different mt haplotypes sampled at locations in Southern Germany, where wasps with different mt haplotypes occur syntopically, exhibit fixed differences in more than 17,000 sites of their nuclear genomes, feature distinct CHC profiles, and subtly differ in their wing morphology from each other. Our results clearly suggest that *P. dominula* represents a conglomerate of two biological species. What evolutionary forces contributed to the reproductive isolation of the two species (e.g., *Wolbachia*) and whether morphometric differences exist that allow identification of museum specimens of the two species (incl. syntypes of *P. dominula*) are currently studied by us in an integrative approach using bioinformatic, molecular, and µCT scanning techniques.

A reevaluation of the diffuse coevolution model in *Trachymyrmex* and *Mycetomoellerius* ants

Jon Seal

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Fungus-gardening ants are often used as examples of 1:1 coevolution; however, this is especially true only at higher phylogenetic levels, i.e., when comparing clades of ants to clades of their symbiotic fungi. However, within clade, where horizontal exchange (symbiont sharing) is thought to be more common, a diffuse (many to one) coevolution model may be more appropriate. There have been relatively few intraspecific studies to examine whether coevolutionary patterns are explained by either model. One of the limitations of prior studies has been that phylogenetic weak markers might obscure the evolutionary history of symbiont exchange, so that that diffuse coevolution might be an artifact of poor phylogenetic resolution. In the following study, we conducted co-phylogenetic analyses of three 'higher' *Trachymyrmex* and one *Mycetomoellerius* species using single-nucleotide polymorphisms, that were obtained from whole genomes of both ants and fungus. We also report the results from cross fostering experiments where we forced newly mated queens to grow different clades of fungi. Both cophylogenetic analyses and experiments indicate significant synergisms among clades of fungi and species of ants. As a result, the results do not support a diffuse coevolution model at intraspecific levels.

Behavioural assessment of spatial vision in the common sunfish (*Lepomis gibbosus*)

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Vision is an important sense for many vertebrates and provides the receivers with information about the 3-dimensional space surrounding them. Previous studies and observations have implied that vision also serves as a crucial sensory modality to the common sunfish (*Lepomis gibbosus*) as vision contributes to many biologically relevant behaviors such as prey detection, courtship or territorial behavior. To give an example, the common sunfish bears unique body patterns which are believed to express dominance over rivalling males or to attract potential mates. Thus, a high spatial resolution, especially for the detection of single targets, could be a vital feature of the sunfish's visual system. We set out to investigate single target acuity (STA) for stationary and moving targets in behavioral experiments with common sunfish and contrasted the STA with a classic visual acuity test including gratings. STA was assessed for stationary dots of three different contrasts and full-contrast dots moving semi-randomly at a speed of 3.36 deg/s. Grating visual acuity was determined by presenting pairs of horizontal and vertical gratings of same stripe width. The dots as well as the gratings were presented on LCD-monitors attached to the aquaria from outside. For STA, the sunfish had to indicate the side of the monitor on which the target was presented while, for grating acuity, the sunfish had to choose the horizontal grating as positive stimulus. STA data revealed no significant differences between stationary (mean 0.14 ± 0.02 deg, N=6) and moving STA (mean 0.14 ± 0.002 deg, N=2) for dots with full contrast to the background. Stationary STA slightly decreased when contrast was lowered. Grating visual acuity was determined as mean 2.44 ± 0.15 cycles/deg (N=) with a single stripe subtending 0.22 ± 0.051 deg and was thus slightly inferior to the STA in common sunfish suggesting a higher ecological relevance for the sunfish to detect single targets. The combined results indicate that the visual system allows the sunfish to detect and extract information about stationary and moving prey items or body patterns from distances up to ~4.5 m.

Morphological and hydrodynamic features of a specialized reproductive strategy in Sulawesi ricefishes (Beloniformes; Adrianichthyidae)

Tobias Spanke; Leon Hilgers; Jana Flury; Mariam Gabelaia; Arne W. Nolte; Leandra Hamann; Ilham V. Utama; Benjamin Wipfler; Fabian Herder; Bernhard Misof; Julia Schwarzer

Affiliation of presenting author: Zoological Research Museum Alexander Koenig

Alterations in an organism's body plan can set the path for a species to survive in a new or changing environment. When multiple adaptations interact with each other, they may form complex character sets that can greatly affect a species' life-history. In ricefishes, a complex reproductive strategy called 'pelvic-brooding' evolved in three species from two distinct lineages endemic to the island of Sulawesi, Indonesia. Usually, female ricefishes spawn every day and attach a few eggs to submerged plants – this is referred to as 'transfer-brooding'. However, pelvic-brooding species carry a large cluster of eggs for about two weeks until the brood hatches. The developing embryos are situated in a ventral concavity and stay attached to the mother via attaching filaments that originate from the eggs. Pelvic-brooding likely evolved from the ancestral transfer-brooding and species from both reproductive strategies differ in morphological, physiological and behavioural traits. Our studies utilize high-resolution μ CT-imaging and 3D morphometrics to investigate the morphological adaptations associated with pelvic brooding. Furthermore, we use computational fluid dynamics in order to determine whether some adaptations in female pelvic-brooders increase drag or lead to a non-streamline shape. Our analyses support highly convergent female-specific adaptations of ribs, pelvic fins and overall body shape in both lineages of pelvic-brooding ricefishes. However, morphologies of pelvic girdles seem to be different, suggesting lineage-specific adaptations to at least some extend. The fluid dynamic simulations reveal that under certain circumstances the ventral concavity can cause turbulences and regions of increased drag in female pelvic-brooders. We hypothesize that strong ecological selection pressures led to these female-specific adaptations connected to pelvic brooding.

The emergence of ecotypes in a parasitoid wasp: A complicated matter!

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To understand which reproductive barriers initiate speciation is a major question in evolutionary research. Despite their high species numbers and specific biology, there are only few studies on speciation in Hymenoptera. The present study aims to identify very early reproductive barriers in a local, sympatric population of *Nasonia vitripennis* (Walker 1836), a hymenopterous parasitoid of fly pupae. It belongs to the Chalcidoidea, one of the most species rich taxa within the Hymenoptera. We studied ecological barriers, sexual barriers, and the reduction in F1-female offspring as a postmating barrier, as well as the population structure using microsatellites. Specifically, we aimed to test the hypothesis by Askew (1968), that inbreeding might be a major driver of speciation in Chalcidoidea. From the literature, it is known that inbreeding is common in *N. vitripennis*. We found a population structure with either three or five subpopulation clusters defined by microsatellites. In addition, there are two ecotypes, one parasitizing fly pupae in bird nests and the other on carrion. The nest ecotype is mainly formed from one of the microsatellite clusters, the two or four remaining microsatellite clusters form the carrion ecotype. There was slight sexual isolation and a reduction in F1-female offspring between inbreeding strains from the same microsatellite clusters and the same ecotypes. This supports the hypothesis, that chalcid wasps form inbreeding lines, which are separated from each other. Strains from different microsatellite clusters are separated by a reduction in F1-female offspring. Ecotypes are separated only by ecological barriers. This is the first demonstration of very early reproductive barriers within a sympatric population of Hymenoptera which might have been caused by inbreeding. It demonstrates that sexual and premating barriers can precede ecological separation. This indicates the complexity of ecotype formation and highlights the general need for more studies within homogenous populations for the identification of the earliest barriers in the speciation process.

Temperature resilience of neuronal activity patterns in the brush-clawed shore crab, *Hemigrapsus takanoi*

Wolfgang Stein; Steffen Harzsch

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The intertidal brush-clawed shore crab, *Hemigrapsus takanoi*, is native to the temperate waters of Japan. It thrives at common ocean salinities and in warm waters, but has recently seen a dramatic invasive expansion into colder European waters. The reasons for this expansion remain unclear. Here, we investigate the temperature range at which the nervous system of *H. takanoi* remains functional as a possible aspect of this species' invasive success. Since the body temperature of intertidal invertebrates closely follows the rapidly changing temperatures in this habitat, neurons are predicted to function over a wide temperature range to ensure survival and facilitate the species' range expansion. We analyzed in vitro temperature responses of the pyloric central pattern generating neurons in the stomatogastric ganglion. The pyloric rhythm serves a vital function in digestion. Like in other decapod species, the pyloric rhythm was continuously active with a triphasic pattern and well-maintained phase relationships ($N > 10$). However, in contrast to other crabs, pyloric rhythm frequency increased only moderately with temperature, reaching a maximum of < 1 Hz below 20°C . Unexpectedly, pyloric phase relationships changed, with warmer temperatures increasing the duty cycle of the pyloric PY neurons. The pyloric rhythm was very cold-resistant. First action potentials failed at $2.7 \pm 0.4^\circ\text{C}$ ($N = 8$). Rhythmic activity stopped at $0.8 \pm 0.3^\circ\text{C}$ ($N = 7$). At high temperature, individual action potentials or bursts failed at $30.5 \pm 0.9^\circ\text{C}$ ($N = 10$). The rhythm crashed at $31.7 \pm 0.9^\circ\text{C}$ ($N = 10$) when neurons started to fire in unpredictable ways. A return to room temperature restored rhythmic activity in all cases. Thus, *H. takanoi* neurons remain functional over a range of almost 30°C . We are currently testing the effects of long-term changes in habitat temperature on neuronal activity.

Yolk formation in a sea anemone provides insights into the evolution of animal nutrient transport

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Circulatory systems play an important role in many bilaterian animals (e.g. vertebrates, flies, annelids) to transport dietary nutrients towards oocytes during yolk formation. Currently, however, only little is known about the evolutionary origin of nutrient transport systems in animals. Here, we have characterized dietary nutrient transport during vitellogenesis in the sea anemone *Nematostella vectensis* (Anthozoa, Cnidaria) as a paradigm to study the evolution of animal nutrient distribution systems. Using a combination of fluorescent bead uptake and expression analysis of key marker genes, we found that the gonad epithelium exhibits increased levels of phagocytosis, micropinocytosis and intracellular digestion of food components. Pulse-chase experiments further show that labelled fatty acids rapidly translocate from the gonad epithelium through the extracellular matrix (ECM) into oocytes. Expression of conserved lipid transport proteins vitellogenin (vtg) and apolipoprotein-B (apoB), and colocalization of labelled fatty acids with an endogenously tagged ApoB-PSmOrange apolipoprotein in the gonad epithelium further support the lipid-shuttling role of the gonad epithelium. In a complementary fashion, we find oocyte expression of very low-density lipoprotein receptor (vldlr) orthologs, conserved in bilaterian Vtg/ApoB-mediated endocytosis of lipids. These findings support that the Vtg ligand/VLDL receptor pair is evolutionary conserved between sea anemone and bilaterians to mediate lipid transport during vitellogenesis. In addition, we identified ECM-based, mesenchymal-like cells with potential role in systemic lipid transport. Altogether, our work supports a long-standing hypothesis that an ECM-based lipid transport system predated the cnidarian-bilaterian split.

Recurring adaptive introgression of a supergene variant that determines social organization

Eckart Stolle

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Introgression has been proposed as an essential source of adaptive genetic variation. However, a key barrier to adaptive introgression is that recombination can break down combinations of alleles that underpin many traits. This barrier might be overcome in supergene regions, where suppressed recombination leads to joint inheritance across many loci. Here, we study the evolution of a large supergene region that determines a major social and ecological trait in *Solenopsis* fire ants: whether colonies have one queen or multiple queens. Using coalescent-based phylogenies built from the genomes of 365 haploid fire ant males, we show that the supergene variant responsible for multiple-queen colonies evolved in one species and repeatedly spread to other species through introgressive hybridization. This finding highlights how supergene architecture can enable a complex adaptive phenotype to recurrently permeate species boundaries.

Alkaline guts contribute to immunity during exposure to acidified seawater in the sea urchin larva

Meike Stumpp

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Larval stages of the ambulacraria superphylum including echinoderms and hemichordates have highly alkaline midguts up to pH 10.5. To date the reason for the evolution of such extreme pH conditions in the gut of these organisms remains unknown. Here we test the hypothesis that analogous to the acidic stomachs of vertebrates, these alkaline conditions may represent a first line of immunity to protect from environmental pathogens. pH optimum curves for five different species of marine bacteria demonstrated a rapid decrease in proliferation rates by 50-60% between pH 8.5 and 9.5. Using the marine bacterium *Vibrio diazotrophicus* that elicits a coordinated immune response in the sea urchin larva, we studied the physiological responses of the midgut pH regulatory machinery to this pathogen. Gastroscopic microelectrode measurements demonstrate a stimulation of midgut alkalization upon infection with *V. diazotrophicus* accompanied by an upregulation of acid-base transporters of the midgut. Pharmacological inhibition of midgut alkalization resulted in an increased mortality rate of larvae during *Vibrio* infection. Reductions in seawater pH resembling ocean acidification (OA) conditions lead to moderate reductions in gastric alkalization. However, these reductions in midgut pH did not affect the immune response and resilience of sea urchin larvae to a *Vibrio* infection under OA conditions. This first set of experiments addressed the evolutionary benefits of the alkaline midgut of ambulacraria larval stages. Our results indicate that alkaline conditions in the gut may serve as a first line of defense against environmental pathogens and that this mechanism can compensate for changes in seawater pH.

Two thirds of all bird species have extensive germline/soma genome differences

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The germline-restricted chromosome (GRC) of songbirds is a peculiar chromosome present in germline cells and absent in somatic cells, and has been found in songbirds ranging from zebra finches to crows. Germline genome data of zebra finch previously showed that the GRC is mainly composed of gene paralogs from the "regular" chromosomes and that these were acquired at different points of songbird evolution. Although recent evidence from nightingale germline genomes suggests that the GRC emerged in the common ancestor of zebra finch and nightingales, how this chromosome evolved during early songbird diversification remains mysterious. With the aim of reconstructing GRC long-term evolution across songbirds, here we sequenced 10x Genomics Chromium draft genomes from germline and soma samples of the same individual from 25 species of Estrildidae and key Passeriformes lineages including two Suboscines. Using testis-specific single-nucleotide variants to determine the GRC gene content, we inferred a highly dynamic history of gene acquisition and loss by the GRC across the phylogeny, with some punctuated dramatic changes. We found only few GRC genes to be ancient and widespread, such as *elavl4* and *cpeb1*, raising the possibility that this enigmatic chromosome might play a role in embryogenesis or gametogenesis. Finally, we provide the first evidence that the GRC also exists in Suboscines, suggesting that two thirds of all extant bird species have extensive germline/soma genome differences.

A global analysis of habitat fragmentation research in reptiles and amphibians: What have we done so far?

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Habitat change and fragmentation is the primary cause of biodiversity loss worldwide. Recent decades have seen a surge of funding, published papers and citations in the field as current threats to biodiversity continue to rise. However, how research directions and agenda are evolving in this field is still poorly understood. In this study, we examined the current state of research on the most threatened groups of vertebrates, reptiles and amphibians. We did so by conducting a global scale review of geographical and taxonomical trends regarding habitat fragmentation (agriculture, logging, fragmentation, urbanisation and roads) and the associated sampling methods and response variables. We reveal a number of biases, in particular that existing research efforts are characterised by distinct continents (e.g. North America, Europe and Australia) and a surplus of studies measuring species richness and abundance. However, we found a shift in research agenda towards studies utilising technological advancements including genetic and spatial data analyses. Our findings suggest important associations between sampling methods and the response variables measured which are commonly used in explaining effects of different types of habitat fragmentation. Increased research investment with appropriate sampling techniques is crucial in biodiversity hotspots such as the tropics where unprecedented threats to herpetofauna exist.

Effects of MHC genotype and relatedness in odour cues of Antarctic fur seals

Jonas Tebbe

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Analyses of immune genes of the major histocompatibility complex (MHC) provide various insights about the immunocompetence of individuals. Many studies have demonstrated the involvement of the MHC in mate choice and survival. Odour cues are also increasingly being recognised as a means of assessing MHC genotype. Likewise, it was shown that MHC gene products can be found in exocrine secretions involved in producing body odours. Hence, individuals may be able to assess relatedness at the MHC via the chemical similarity of odour profiles. In this study, we tested for effects of both the MHC DQB II and the genomic background, measured with 41 microsatellites, on the body odour of Antarctic fur seals using a multi-model inference framework. We did not find any effects of MHC similarity or genetic relatedness on odour similarity, but multilocus heterozygosity and the number of odorants were highly correlated. These findings suggest that odour cues reflect overall genetic quality and possibly play a role in mate choice. Contrary to our expectations, we found no effect of MHC genotype on odour, implying that the MHC may not be important in mate choice in Antarctic fur seals.

Mating strategies in cichlid fishes: on inbreeding avoidance, preference and selfing

Timo Thünken; Simon Vitt

Affiliation of presenting author: University of Bonn

While mating strategies are highly variable in plants ranging from outcrossing to selfing and mixed mating systems, in animals and especially in vertebrates mating systems are assumed to be characterized by outbreeding and active inbreeding avoidance. However, recent meta-analyses suggest that avoidance of inbreeding is rarer than expected in animals, and evolutionary theory also predicts benefits from inbreeding and even selfing. Cichlid fishes show great variation in reproductive strategies and are accordingly highly suitable to study the evolution of mating and breeding systems. We examined the impact of multigenerational inbreeding (i.e., resulting from continuous full-sibling matings) on (i) male and female reproductive performance, (ii) juvenile social behaviour and (iii) the mating behaviour of adults in *Pelvicachromis taeniatus*. This monogamous, biparental cichlid from West Africa has been shown to prefer kin as mating partner. Previous studies showed no evidence for inbreeding depression in F1-inbred offspring. In contrast, in the 4th generation inbred fish showed signs of inbreeding depression at early developmental stage. Outbred crosses between inbred lineages did not differ from crosses between outbred lineages indicating heterosis effects. Also, mating behaviour was altered by continuous inbreeding. While 4th generation inbred fish preferred non-kin, outbred fish showed similar kin preferences as reported in previous studies. Our results suggest that the costs of inbreeding are not constant but change with persisting inbreeding. Also, kin mating preferences seem to change according to the associated costs and benefits of mating with kin. We discuss several mechanisms that may contribute to maintain an optimal level of inbreeding. In the second part of the talk, we provide evidence for self-fertilization in another West African cichlid fish, *Benitochromis nigrodorsalis*. This species usually reproduces sexually and provides biparental brood care. We observed a wild-caught female repeatedly producing viable offspring in the absence of a mating partner. Applying whole-genome sequencing other reproduction modes than selfing could be excluded. Our studies provide promising new insights into the evolution of animal mating systems in general and furthermore highlights the great variability of mating systems in cichlid fishes.

Effect of dietary protein content on innate immunity: a mechanistic and evolutionary perspective

Vaibhvi Vaibhvi; Thomas Roeder

Affiliation of presenting author: CAU Kiel

The most accepted evolutionary explanation for lifespan extension on dietary restriction (DR) is the disposable soma theory, which explains it as increased investment in somatic maintenance when facing nutrient shortage until the resumption of nutrients. However, immunity, a critical somatic trait, shows ambiguous results in response to DR. One reason could be the higher degree of complexity and several layers of components contributing to an immune response. Addressing this issue, we did a comprehensive analysis of various parameters contributing to the immune response under the influence of a restricted diet. We used *Drosophila melanogaster* as a model, as its lack of adaptive response decreases some complexity and helps analyze the innate response in more detail. We standardized a system of different dietary regimes and immune induction in the said system, which resolves the animals on different diets in terms of holistic immune parameters, including survival to the infection and bacterial clearance rate. Surprisingly, we saw no substantial differences in the induction of the most vital immune regulatory genes at the level of the whole animal. We further targeted the two cell types contributing the most to immunity, i.e., fat body and hemocytes (functionally equivalent to the mammalian liver and blood cells, respectively), and did a transcriptomic analysis under an immune induced state in different dietary conditions. We see the same trend of almost equivalent induction of important immune regulators in the different diets post-infection at the tissue level. However, we see that some pathways that are probable indirect contributors to the immune reaction and are induced in the control state on bacterial infection (including but not limited to redox homeostasis, proteasomal machinery, lipid, carbohydrate, and protein metabolism) are not regulated to the same extent in the nutritionally restricted diet. We also observe that the concerned pathways are different in the different cell types. Altogether, our study suggests that DR affects resource allocation in the immune response as well, but not in terms of the major immune contributors but rather the indirect cell maintenance strategies that are also essential for fighting the infection.

Effects of heat-shocks on sociometry in the invasive ant species *Cardiocondyla obscurior*

Esther van den Bos; Jürgen Gadau; Lukas Schrader

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Insect sociometry, the quantitative description of colony attributes (Tschinkel, 1991), describes the most basic superorganismal traits such as colony size, colony age, worker size distribution, or seasonal cycles. Different sets of such traits are often aggregated into sociometric syndromes, given strong degrees of correlation and coevolutionary signatures. The increase in individual worker size over the lifetime of a colony has predominantly been associated with colony growth in terms of worker numbers, as average worker size and colony size are usually highly correlated. Here we show that in *Cardiocondyla obscurior*, an invasive ant species with monomorphic workers, worker size increases over time, independent of colony size. Instead, our findings suggest that queen age or average age of adult workers in a colony determine average worker size produced by a colony. Additionally, we show that this association is disrupted when colonies are exposed to regular stress, as worker size remained constant when colonies were exposed to heat-shock twice a week. Together, these findings suggest that 1) the physiology and life history of the queen or workers and not colony demography alone are determinants of worker size, and that 2) this association is highly susceptible to environmental stress. Whether this is an intrinsic mechanism, i.e. the stress effected the larval physiology and growth directly, or an indirect effect that the brood care of stressed or younger workers influenced the growth through a different brood caring scheme is unclear.

InsectMow: Development and evaluation of insect- and spider- friendly mowing techniques

Lea von Berg; Manuela Sann; Jonas Frank; Oliver Betz; Johannes Steidle; Stefan Böttinger

Affiliation of presenting author: Eberhard Karls Universität Tübingen

A number of studies demonstrate that large amounts of insects and spiders living on agricultural grassland are damaged and/or killed during mowing. Thus, it is safe to assume that frequent mowing of intensively used grassland is analogous to overfishing and significantly contributes to the current insect decline in agricultural areas. The interdisciplinary project „InsectMow“ aims to study how negative effects of mowing on grassland ecosystems can be reduced by technically modifying standard grass mowers. Our main project goals are the development of (a) an insect scarecrow that is mounted in front of the mower to chase away insects and (b) an optimized, arthropod-friendly rotary mower that damages/kills significantly lower numbers of insects and spiders during operation. We study important pollinators (e.g. wild bees, hoverflies, butterflies), herbivores (crickets, grasshoppers, true bugs, some carabid beetles), carnivorous insects (carabid beetles, rove beetles) and spiders. When manufacturers of mowing machines include our tested and approved modifications in their assortment, the project will considerably contribute to the conservation of biodiversity and the maintenance of ecosystem services in agricultural fields, with only limited impact on productivity.

Active pre-touch sensing: from biology to neuromorphic biomimetic artifacts

Tim Walther; Jacob Engelmann; Elisabetta Chicca; Gerhard von der Emde

Affiliation of presenting author: Bonn University

Electric field pre-touch sensors are devices inspired by the perceptual channel of weakly electric fish. Fundamentally measuring capacitance, such devices can provide information on objects in the local environment such as object geometry, position, and a motion parallax-like cue. Similar to sensory inputs faced by electric fish, signals embedded in the carrier wave of pre-touch sensors tend to be noisy. Motivated by the need to extract relevant information on the electric environment, a faithful model of the electrosensory lateral line lobe (ELL) based on physiological data was constructed and will be presented in this talk. The functional characteristics of the connectivity reproduce the known characteristics of the ELL in that the neuronal circuit of the model is essentially a feed-forward network with a somatopic arrangement. Local electrosensory input patterns are converted into a latency code by primary sensory afferents, gated by the electric organ discharges (EOD) and modulated by descending feedback driven by the electric organ corollary discharge (EOCD) in subsequent stages. Processed by a mixture of overlapping receptive fields, spatial summation, parallel information streams, and lateral inhibition; spatio-temporal information is eventually relayed by a small population of feature selective neurons in the final compartment of the network. Using methods from detection and information theory, it will be illustrated that the first stages of sensory processing in electric fish are specialized on detecting peaks in the input. The overall focus of this talk lies on biological particularities of neuronal computation of information in the sub-millisecond regime, especially how filtering and amplification contribute to the improvement of frail signals. The presented model is however also highly suitable for an implementation in analog neuromorphic hardware. Respective applicability is ensured by neuronal and synaptical models that reflect the realistic behavior of analogous devices in silicon. The here presented model can therefore be used in conjunction with electric field sensors, for example in robotic contexts, with sensors either operating in air or aquatic media.

Strategic pheromone signaling by females of the sexually cannibalistic spider *Argiope bruennichi*

Katharina Weiss; Jutta Schneider

Affiliation of presenting author: Freiburg University

Finding a mating partner constitutes one of the costs of sexual reproduction. In many arthropods, receptive females advertise their presence to roving males via volatile chemical signals. These pheromones are traditionally considered low-cost signals due to the often minute amounts released and their production and transport requiring little energy. This notion, however, is based on little empirical evidence and challenged by recent studies. On the other hand, signaling females face the risk of failing to attract a male, a common, yet often underappreciated problem for female arthropods. Hence, selection should favor females that modulate their investment in signaling relative to potential costs such as the perceived risk of mating failure. We tested this strategic-signaling hypothesis using the orb-web spider *Argiope bruennichi*. In this species, egg maturation is independent of a female's mating status and also virgin females will produce a first unfertilized egg sac about three weeks after maturation. As females produce only two egg sacs on average, this constitutes a considerable fitness loss. Moreover, males are protandrous and their number rapidly declines over the course of the short mating season mainly due to post-copulatory sexual cannibalism. Thus, with every day a female remains unmated, the risk of mating failure increases. We combined chemical analyses and behavioral assays to investigate the condition-dependence of female signaling effort. In support of the strategic-signaling hypothesis, we found virgin females to intensify pheromone signaling with increasing age as well as body condition, thereby increasing their attractiveness for males in binary choice tests. As especially female body condition is a crucial predictor of fecundity in spiders, we argue that the pheromone of *A. bruennichi* females qualifies as an honest signal facilitating adaptive male mate choice.

Love your Data!

Jeanne Wilbrandt

Affiliation of presenting author: Leibniz Institute on Aging - Fritz Lipmann Institute

Research Data Management is gaining traction as the talk of town in Germany: funding agencies start to demand Data Management Plans, the NFDI is taking up their work to connect research infrastructures, data stewards are hired. With this talk, I would like to raise awareness for this topic among researchers and provide them with terms and motivation to talk about and improve their data handling. Questions to ask yourself here are for example „How do I name my files so I can find them again?“, „What does this variable name stand for?“, or „Will I know in 6 months time how I measured this parameter?“. Answers to these and many more are required to write a Data Management Plan and thus ultimately to acquire funding. Here, I will outline the basic concepts, benefits, and resources available to setup and maintain good data management in compliance with good scientific practice. Remember: Documenting your research is like writing a love letter to your data. Love your data!

A quantitative 3D gut atlas of the model insect *Manduca sexta*

Anton Windfelder; Gabriele Krombach; Marian Kampschulte; Andreas Vilcinskas

Affiliation of presenting author: Fraunhofer-Institut für Molekularbiologie und Angewandte Oekologie IME Institut

The tobacco hornworm (*Manduca sexta*) is of extraordinary significance for numerous life science disciplines. It is used as a model in ecotoxicology and agricultural science but also in basic immunology, gastroenterology and gut physiology. Standard histology is a powerful tool to reveal the anatomy in two dimensions. However, most structures and phenotypes have a three-dimensional character. We established a new contrast method based on the oral application of the clinical contrast agent iodixanol. With that, we quantified the alimentary tract of *Manduca sexta* in exceptional detail via micro-CT. This approach bypasses chemical fixation and avoids soft-tissue shrinkage for accurate quantification. With oral iodixanol-contrast, we show previously unknown structures in *M. sexta* like the gastric caeca and reveal the unknown complexity of the hindgut folding pattern, which is involved in fecal pellet formation. Here we provide a systematic, morphological and quantitative reference atlas of the digestive tract of *M. sexta*. We expect this atlas to be a reference for calculating locally effective midgut concentrations for applied chemicals. In addition, it will support the problematic orientation within histological sections, enable quantitative histological 3d-phenotyping and allow comprehensive animal screenings, giving insights into the structural evolution of the alimentary tract in lepidopterans and other insects.

Analysis of spatial differences in chromatin accessibility and enhancer activity using ATAC-seq and live imaging in the red flour beetle *Tribolium castaneum*

Christine Zellner; Heike Rudolf; Frederic Strobl; Ernst Stelzer; Timo Regensburger; Benjamin Schmid; Leila Taher; Ezzat El-Sherif

Affiliation of presenting author: Friedrich - Alexander - Universität Erlangen - Nürnberg

Patterning by waves of gene expression seems to be a widespread mechanism in development. Such gene expression waves are initiated as a broad expression domain that propagate, shrink while propagating, and then stabilize as defined expression domains. Therefore, gene regulation switches from a dynamic behavior (initialization of gene expression) to a static behavior (stabilization of gene expression). In a recent study (Zhu X, Rudolf H, Healey L, François P, Brown SJ, Klingler M, El-Sherif E., 2017, Proc Natl Acad Sci U S A.), this behavior was explained by an ‘Enhancer Switching Model’ implementing a set of dynamic enhancers that initialize gene expression and static enhancers that stabilize gene expression. Here, we identify such enhancers using computational predictions and genomics data in *Tribolium*. Activity dynamics of putative enhancer regions were examined using reporter assays (*in situ* and in live *Tribolium* embryos using the MS2-MCP system) and time- and tissue-specific ATAC-seq data. The obtained results are in line with the Enhancer Switching Model.

Are centipedes heavy metal? Investigation of cuticular components and metals in the centipede forcipule

Simon Züger; Andy Sombke; Harald Krenn

Affiliation of presenting author: University of Vienna

Centipedes are known for their fearsome appearance due to their peculiar forcipules that are used in grabbing, restraining, and poisoning their prey. As their active use exposes them to strong mechanical forces, we hypothesise that especially the distal cuticular components are reinforced. It is known from many arthropods that they incorporate heavy metals into the cuticle of piercing or biting structures, thus increasing their hardness and elasticity. In the mouthparts of different insects, metals like zinc, manganese, iron, and calcium are present in the biting edges of mandibles. In spider chelicerae, or the scorpion stinger, zinc and calcium are embedded to withstand wearing off. Centipede forcipules are evolutionary transformed locomotory legs which have venom glands with an opening at the pointed tip. However, not much is known about the metals and other cuticular components that strengthen these structures. To investigate these properties, we used energy-dispersive X-ray to examine elemental components in the cuticle of representative species of all five centipede orders (Scutigeromorpha, Craterostigmomorpha, Lithobiomorpha, Scolopendromorpha, and Geophilomorpha). Furthermore, cuticular hardness gradients were analysed using confocal laser-scanning microscopy based on autofluorescence properties. Additionally, we compared the forcipules with the serial homologous locomotory legs, to test whether this reinforcement only occurs in the weaponized forcipules or is a general feature in the distal structures of these appendages. Finally, our results for individual species and higher taxonomic groups are compared to gain a deeper understanding in taxon-specific modifications and environmental interactions.

Posters

(Sorted by last name of presenting author)

Investigation of the diversification of ant-associated Lycaenidae butterflies during the aridification of the African continent.

Svenja Sabine Ahlborn; Marianne Espeland

Affiliation of presenting author: Zoologischen Forschungsmuseums Alexander Koenig (ZFMK), Bonn

Over the past 20 Myr the African continent became increasingly arid. This major environmental change had massive impact on the flora and fauna, leading both to radiation and extinction of species. The mechanisms that have driven the diversification during that time are not fully known yet. However, some life histories may have favored an explosive diversification of some species groups, such as the ant-association found in a variety of forms in the Lycaenidae butterflies. Indeed about 75% of all Lycaenidae butterflies (about 6,000 species) have an ant-associated behavior which can vary from a facultative mutualistic relation to an obligate and parasitic relation. It is hypothesized that ant nests present a shelter from fires and desiccation and provide a stable food source in this otherwise arid environment. The effects of ant-association on the morphology, the niche evolution, and the species diversification have not yet been intensely studied. A recent phylogenetic study, by Espeland et al. (submitted), of the genus *Lepidochrysops* supports the hypothesis that a massive diversification arose through the evolution of the ant-association (around 6.5 Mya) as an adaptive response to the aridification of Africa. Thus, allowing the survival of the genus in an increasingly arid environment and giving it the opportunity to spread into drier regions. Considering this previous study, my PhD project aims to investigate that hypothesis in a broader frame, including more genera: *Lepidochrysops* and its closest relatives *Euchrysops*, *Oboronia*, *Thermoniphias*, and *Orachrysops*. The data of the project relies on museum specimens, from a variety of different institutions around the world. It will include classical taxonomic descriptions, using macrophotography and stereo microscopy, to identify the species of the five genera and revise their taxonomy. Using the gathered collection localities of the specimens, biogeographic data for the species will be gained and used to assess the distribution and ecological niches of the species. Through DNA analysis the phylogeny of the genera will be studied to get more insights into their diversification under the impact of the aridification of Africa, taking into account the ant-association of the species. All the gathered data will then be used for a taxonomic and phylogenetic revision of these five genera and get some more clarification of key mechanisms driving diversification of species on the African continent.

Logarithmic claws – quantifying claw shape in various lineages of Bilateria

Pia Althoff; Vanessa Fischer; Joachim T. Haug

Affiliation of presenting author: University of Munich (LMU)

The “spira mirabilis” better known as the logarithmic spiral is all time present in our daily life. Most of us will not even notice it and the beautiful mathematical concept behind things we see as given by default. The best-known form is the Fibonacci Spiral which finds use in the astonishing artworks of many artists. Since nature is the greatest artist of all time, it is no surprise that it also uses the logarithmic spiral for its formations. The spiral, for example, is found in the arrangement of sunflower seeds, the curvature of nautilus shells, and the architecture of spiderwebs. The unique property of the “spira mirabilis” is its tangential vectors. These vectors create a tangential angle with the intersection point of the radianc vector and the spiral. This angle is the same along the entire spiral. Here, we use the mathematical basics of the logarithmic spiral to analyze and quantify claw shapes within polar coordinates. All claws are placed at the same point inside a polar coordinate system. With fixed radianc vectors (vectors that start at the origin of the system and cut through the inside and outside curvature of the claw) the measurements change in the height and angle of the claw and give the opportunity for comparison. The first run through this method compares well-known flesh-piercing claws from Euarthropoda, Ornithodira, and Pantherinae. The procedure of hunting down and piercing the flesh of prey to hold on to it may be the reason for the claws to grow logarithmic. If a claw is curved along the logarithmic spiral it reaches the point of the highest resistance against force vectors. Force vectors come into effect when claws are pierced into the flesh of a struggling prey animal. The probability of claw damage is lowest with a logarithmic curved claw. The logarithmic spiral provides a comparative frame for only distantly related organisms with fixed points and angles.

Impact of dot patterns on mate choice in a cichlid fish

Christina Bakowski; Simon Vitt; Timo Thünken

Affiliation of presenting author: University Bonn

In fishes, there is high variation within and between species in body coloration and ornamentation. Colour patterns have been proposed to play an important role during intra- and inter-sexual communication. The West African cichlid *Pelvicachromis taeniatus* shows conspicuous body coloration and dot patterns of the caudal fin in both sexes; especially the males greatly vary in the number of dots. Here, we investigated the impact of male dot patterns on female mate choice. To exclude other potentially influencing variables, such as coloration and size, we used computer-animated males that differed only in the number of dots on their caudal fin. The presented males had either two or twelve dots on their caudal fin. The animations were presented on tablet computers, with the animated males swimming from side to side several times. Two animations were presented simultaneously on opposing sides of the experimental tank. The courtship behaviour of the females was examined by quantifying the time spent in association zones close to the males. *P. taeniatus* prefer kin as mating partners and dot patterns may play a role during kin recognition as they differ between families. Accordingly, females are expected to prefer males showing a similar number of spots as their brothers. Alternatively, males with a higher number of dots could be preferred generally, assuming that the number of dots is linked to quality, e.g., physical condition or fertility.

Out of Africa? The biogeographic origin and radiation of Malagasy stick insects

Sarah Bank-Aubin; Sven Bradler

Affiliation of presenting author: Georg-August Universität Göttingen

The stick insects of Madagascar exhibit a high level of diversity and disparity, in particular in regard to body size and colouration. This variety was traditionally attributed to a vicariance hypothesis where the Malagasy stick insects represent several distinct subgroups belonging to unrelated phasmatodean lineages that are mainly found in other tropical regions of the world. More recent studies indicated that their diversity may rather be the result of adaptive radiation following the colonisation of the geographically isolated island of Madagascar through long-distance dispersal. It remains unclear from where such dispersal may have taken place. Since the monophyly of the group has also never been explicitly explored, multiple independent dispersals cannot be ruled out. In order to investigate the phylogenetic relationships and the historical biogeography of Malagasy stick insects, we conducted phylogenetic and divergence times analyses using Sanger sequence data of 122 stick insects from Madagascar and the Comoros, 20 from Africa and 16 from the Mediterranean (plus several outgroups including all major phasmatodean taxa) in combination with a selection of >100 loci from previously published transcriptomes. Our results support the Malagasy stick insects to form a natural group that originated from a single colonisation event from Africa to Madagascar and that underwent extensive and rapid diversification. The Comoro islands – though situated between Africa and Madagascar – were not found as part of a stepping stone dispersal but were secondarily colonised from Madagascar and remain the only landmasses to which the Malagasy stick insects have dispersed. Our analyses also identified multiple undescribed species depicting the vast unexplored biodiversity of Madagascar that needs yet to be uncovered.

UV-dependent habitat choice in parasitized and non-parasitized *Gammarus pulex*

Sebastian Bauer, Timo Thünken, Gerhard von der Emde, Ingolf Rick

Affiliation of presenting author: University of Bonn

The common amphipod *Gammarus pulex* is a freshwater crustacean found in Europe and central Asia. *G. pulex* is an important food source for various fish and bird species. Some parasite species take advantage of this by using amphipods as intermediate hosts in order to reach their final hosts. For instance, certain acanthocephalan parasites, such as *Polymorphus minutus*, are able to change the phenotype and behavior of *G. pulex* as intermediate host in order to increase the chance of being ingested by a final host. The present study aimed to investigate whether ambient UV-radiation as an abiotic stress factor has an impact on the host-parasite interaction between *G. pulex* and *P. minutus*. In a lab experiment under full-spectrum illumination conditions, individual *G. pulex*, being either *P. minutus* infected or uninfected, were given the choice between two light habitats differing in spectral UV-content (UV+: 300-700 nm, UV-: 400-700 nm). A preference index was calculated for each individual based on the relative time spent in the two light habitats. In addition, total activity was determined as the number of vertical zone changes independent of light habitat. Infected gammarids showed a significant UV-avoidance, i.e. they spent less time in the UV+ habitat, at least during the first half of the experimental trials. In contrast, uninfected gammarids did not show any light habitat preference or avoidance. Moreover, total activity during the experimental trials was not significantly different between infected and uninfected gammarids. Our findings are discussed in the light of responses to UV-induced stress in hosts and parasites and associated effects on host-parasite interactions.

Composition and function of chitin-synthesizing supercomplexes from the elytral cuticle of the red flour beetle, *Tribolium castaneum*

Marius J. Beck; Hans Michael Merzendorfer

Affiliation of presenting author: Universität Siegen

Chitin is a polymer of $\beta(1,4)$ -linked N-acetylglucosamines produced by a membrane-bound glycosyltransferase termed chitin synthase. It is produced by many organisms from diverse taxonomic groups (e.g. fungi and insects) for the construction of various types of extracellular matrices including cell walls, cuticles, and peritrophic matrices. Insect cuticles serve as exoskeleton and protect from desiccation and microbial infections (Muthukrishnan et al. 2019). Peritrophic matrices cover the midgut epithelium of most insects, increase digestive performance, and have functions in antimicrobial defense (Agrawal et al., 2014, Kuraishi et al., 2011). Given that chitin biosynthesis has essential roles in insect growth and development, it makes this process a potential target for new and sustainable insect pest management strategies in agriculture. Chitin and its deacetylated derivative chitosan are becoming increasingly important for industrial, biomedical and pharmaceutical applications. Existing natural chitin sources yield heterogeneous chitin preparations, which impede applications requiring homogenous polymers. To exploit the versatile potential of chitin and chitosans the precise mechanisms of biosynthesis have to be elucidated and made applicable in a controlled manner including the use of heterologous expression systems. Our future work will investigate hitherto hypothetical chitin-synthesizing supercomplexes, which potentially combine catalytic and non-catalytic functions in chitin biosynthesis and modification and fibrillogenesis. We will conduct these studies on the red flour beetle, *Tribolium castaneum*, which is a major pest of stored cereal products and due to the availability of a fully sequenced genome and systemic RNA interference an important insect model in research. We will identify proteins interacting with chitin synthase 1 from pupal elytra and investigate their function in regulating chitin synthase activity, chitin modification and fibrillogenesis. We will present the first bioinformatic approaches to identify potential interaction partners.

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Effects of ambient temperature on the locomotion of juvenile American cockroaches

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Locomotion on legs is flexible in terrestrial animals and can be adapted to the requirements of the respective situation. Due to changes in the legs' temporal coordination, locomotion dynamics can change drastically when animals transition from slow to fast speeds. So far adaptations of the leg coordination patterns and body dynamics have rarely been analysed in the context of environmental constraints, as have the effects of the developmental stage. With respect to the biomechanics and physiology of running, the American cockroach is one of the best examined species and generally a well-established laboratory species. The originally subtropical species is characterized by long legs and its ability for fast sprints. For adults it has been shown that the stride frequency increases linearly with speed. On level ground, they typically use tripod coordination, while metachronal coordination is observed primarily when they move slowly. In the present study we investigate the influence of different ambient temperatures on the locomotion of juvenile American cockroaches with body length of about 10 mm, i.e. approx. 1/3 of adult's body length and 1/10 of their weight. The upper and lower temperature limit at which escape runs could be elicited were 45°C and 20°C. These temperatures were used to examine physiologically constrained locomotion. The third temperature at which the animals were examined was 30°C, which corresponds to their natural habitat. A range of kinematic and dynamic parameters such as stride frequency, swing and contact duration, the distance the COM travels during a stride and the duty factor, was analysed for the entire speed range of all three temperatures. The stride frequency and speed were shown to increase with the rise in temperature, reaching values of up to 40 s^{-1} and $0,75\text{ ms}^{-1}$ at 45°C. In contrast to published data on adult specimens, the juveniles showed biphasic changes, from tripodal to metachronal and back to tripodal leg coordination, which also clearly influenced the body dynamics. The metachronal leg coordination occurred mainly at medium speeds and resulted in dominating horizontal body oscillations. The change probably exploits increased higher dynamic stability against lateral disturbances. The presence of tripod coordination at the highest speeds can be explained due to the high peak total forces required to achieve ballistic phases and longer strides.

Influence of a RVFV infection on the olfactory perception and behaviour in *Drosophila melanogaster*

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In blood-feeding dipterans, olfaction plays a role in finding hosts and hence spreading pathogens. Several pathogens are known to alter olfactory responses and behaviour influencing vector competence during infection. As a mosquito-borne pathogen, Rift Valley Fever Virus (RVFV) can affect humans and cause great losses in livestock. It would be advantageous to study potential effects of human pathogenic viruses on insect sensory perception and behaviour on a non-biting insect, like *Drosophila melanogaster*. We test the influence of RVFV infection on electroantennograms (EAG) and olfactory choice behaviour via Y-maze on the wild type *Drosophila* strain CnBw, using different food related odors as stimuli. Flies were injected intrathoracically with a dose of 1000 FFU of RVFV MP12 strain in 9.2 nl. Replication of RVFV and its persistence for at least 7 days could be confirmed by qPCR. Mock injected flies served as uninfected controls. In uninfected flies, apple cider vinegar and 1-hexanol yielded strong EAG responses, whereas ethyl acetate resulted in moderate responses. This was reflected by corresponding response indices (RI) in Y-maze experiments. Flies strongly preferred vinegar and 1-hexanol to controls. Ethyl acetate failed to produce a detectable response. One day post injection, infected flies showed weaker EAG responses towards 1-hexanol, vinegar, and ethyl acetate. In the Y-maze, infected flies showed a significantly lower RI for 1-hexanol compared to control flies, whereas no significant effect was detectable for vinegar. At days six or seven post infection, no significant difference between infected and control flies could be found in the EAG or in the Y-maze anymore. A possible target for modulation of olfactory perception is the co-receptor Orco, which can be inhibited by its specific antagonist, BMP. High doses decreased responses to 1-hexanol and other odors in the EAG. In the Y-maze, BMP in combination with 1-hexanol still resulted in high attraction compared to controls, but decreased the decision-making. Our results show that an infection with RVFV holds the potential to transiently reduce olfactory perception and attraction towards food related odors in *Drosophila*. A similar effect in blood-feeding insects could affect vector competence in RVFV transmitting dipterans. We now address potential effects of viral infection on overall activity using a locomotor activity monitor.

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A calcification model of a rotaliid foraminifera based on live imaging data of its autofluorescence combined with dye specific staining of different vesicles

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Biologically controlled mineralization is of key importance in development of new technologies in materials engineering inspired by biological solutions at macro to nanoscales. Besides, production of biologically induced carbonates is one of the most important phenomena driving global carbon circulation. Unfortunately to date many of the fundamental problems of biomineralization have not been resolved. Here we present a model in understanding of biocalcification process in *Amphistegina foraminifera* by *in vivo* confocal autofluorescence and dye fluorescence imaging in combination with electron microprobe determination of carbonates inside the cells of foraminifera. Foraminiferal shell is produced via intracellular formation of Mg-rich amorphous calcium carbonate (Mg-ACC) precursor. This process is accompanied by activity of endosymbiotic microalgae and seawater-derived endocytic vesicles providing calcification substrates CO₂, and Ca and Mg ions respectively as well as vesicles controlling pH homeostasis. Completed Mg-ACCs release their content into nanoportions that are subsequently distributed by the cytoskeleton to the place of the shell construction where Mg²⁺ ions are liberated as measured by Mag-Fura2. Our studies address the understanding of physiological processes involved in marine biocalcification mechanism in general.

Single-cell analysis of *Drosophila* larval trachea

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The tracheal system of the fruit fly *Drosophila* is well studied regarding processes like organ development and used for the analysis of chronic human lung diseases like asthma and COPD. It has to fulfill a number of different tasks with gas exchange being the most important one. The passage of two different molting stages and the following development into the adult tracheal system requires different cell types and adaptations of the larval tracheal system. Nevertheless, a comprehensive study of all cell types and their underlying molecular setup is still missing. To fill this gap, we performed single-cell sequencing with the entire tracheal system. We used different antibodies and reporter lines for marker genes to identify the different cell populations and assign them to the corresponding single cell cluster. Despite the different populations of trunk cells and stem cells, we could also identify the existence of several smaller cell clusters which we think could correspond to a multicellular epitracheal organ. One cell cluster belongs to the already known Inka cell which is characterized by the expression of the ecdysis triggering hormone (ETH). Two other cell clusters share the expression of the same marker gene, while another cell cluster is marked by the expression of a different marker gene. All three clusters are localized in proximity to the Inka cell. On the other hand, we focused on the characterization of different types of stem cells. Three groups of progenitor cells can be distinguished in the larval trachea, the spiracular branch (SB) tracheoblasts, the cells of the air sac primordia (ASP), and transformed epithelial cells, which become progenitors for the adult airway system at a specific developmental stage. These different progenitor cells give rise to the entire adult tracheal system. By our analysis we could shed light on still unknown cell types of the tracheal system as well as on differences on supposedly similar cell types.

Immunohistochemistry of spinal central pattern generators in the African mudskipper *Periophthalmus barbarus*

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Mudskippers are amphibious fish that make use of different motor programs for locomotion under water and on land. When swimming, propulsion is mainly generated by undulatory body motions along the axial musculature, with the pelvic fins pulled against the body and pectoral fins mainly used for steering. When moving on land, the axial musculature seems to not be substantially involved in locomotion. Instead, pectoral and pelvic fins are used to produce a well-coordinated, "crutching" locomotor pattern. We aim to understand the spinal neural networks (CPGs) that control these vastly different kinds of locomotor behaviors. Here, we present staining of spinal cord (SC) motoneurons (MNs) through in-vitro nerve backfills. These backfills were combined with an immunohistochemical study to characterize the neurochemical profile of cells that compose the CPGs for pectoral, pelvic and axial muscle control. Backfills of pectoral and pelvic nerves and axial ventral roots with the low-molecular weight tracer neurobiotin in combination with dextran-coupled dyes of higher molecular weight (Dx-Fluoresceine and Dx-Rhodamine, 3-10 kDa) led to transneuronal staining of pre-motoneurons in the mudskipper SC. Immunohistochemistry (IHC) against connexin 35/36 revealed gap junctions located on MNs, indicating that the transneuronal staining was due to gap junction passage of neurobiotin. IHC against vGLUT2 revealed a population of medially located interneurons. Backfilled MNs were vGLUT2 negative. However, a second population of large neurons located slightly more lateral to the backfilled MNs showed a clear vGLUT2 signal. Tyrosine hydroxylase positive fibers were located in the dorsal parts of the SC, and were missing around the MNs. The ongoing investigation of the neurochemical profiles of the CPGs that control pectoral, pelvic and axial muscle activity in mudskippers, combined with electrophysiological experiments, will provide insights into the activity and interconnectivity of these networks. This will help to decipher the necessary adaptations of spinal CPGs that allow mudskippers to perform novel motor behaviors for locomotion on land.

Epigenetic modification during diapause in *Daphnia magna* embryos

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Organisms have evolved different strategies to overcome periods of adverse environmental conditions. For example, diapause, which occurs during a specific developmental stage. Diapause is usually characterized by arrested ontogenesis, decreased metabolic activity, and increased stress resistance. These diapause-hallmarks may result from changes in gene expression. There is growing evidence that development-related genes are downregulated while diapause-related genes are specifically upregulated during diapause. This raises the question as to what governs distinct gene expression patterns during diapause. Epigenetic mechanisms which influence the gene expression without accompanying alternations in the DNA sequence, have been suggested to play a role in regulating gene expression during diapause. We investigated the role of histone modifications and DNA methylation, which are the two main mechanisms of epigenetics during diapause development in the water flea *Daphnia magna*. We analyzed the expression patterns of genes encoding histone methyltransferase (*suv4* and *suv39*) and DNA methyltransferase (*dnmt1* and *dnmt3*) at different stages of *D. magna* diapause destined embryos using quantitative PCR. Our results demonstrate that *suv4* and *suv39* decreased during diapause. During resurrection the expression level of these genes increase, indicating that they probably play a subordinate role during diapause phase but a more significant role in active development. In contrast, *dnmt3* is constantly expressed even during diapause, which may suggest its involvement in diapause maintenance through constant gene silencing activity. Our study shows that DNA methylation plays a crucial role during diapause of *Daphnia magna*. Furthermore, the investigated histone methyltransferases appear to be essential for diapause-determined development in the pre- and post- diapause phases.

Evolutionary genetics of convergent mimicry color patterns in bumblebees

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Convergent evolution of complex phenotypes is one of the most striking and fascinating examples of adaptive evolution. Shaped by natural selection, unrelated organisms can converge on an surprising level and mimic each other in color and shape, often to send the same aposematic signals to prevent predation (Müllerian mimicry). However, the molecular underpinning of these striking case of evolutionary convergence are not well understood. A group of important pollinators, bumblebees, have convergently evolved several color patterns which are shared across unrelated bumblebee lineages. Strikingly, some polymorphic species show similar intraspecific color pattern shifts parallel to other, unrelated sympatric bumblebee species. These species provide an excellent opportunity to study the genomic architecture of these adaptive phenotypic traits as well as their convergent evolution. Due to the enormous technological advances a broad comparative genomics approach combined with developmental genetics is now feasible and will provide comprehensive insights in the genetic mechanisms facilitating and regulating color shifts in bumblebees, but also whether convergent shifts are underpinned by the same mechanisms, or stem from different molecular changes. In my project I investigate four independent shifts (four unrelated polymorphic species) between the two predominant color patterns in European bumblebee species (black-yellow-white to black-red) and include cases of intra-specific convergence and parallel color shifts in host bumblebees and the associated social parasite. The study will shed light on how genomic architecture leads to adaptive color polymorphisms within and between species and is facilitated by genome structure, coding sequence differences or regulatory change.

Brain activation patterns in cichlid fish during different behavioral contexts

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Fish have been the subject of many visual discrimination experiments showing that fish possess cognitive abilities equaling those of birds and mammals. Unfortunately, only few studies have described where and how cognitive information is processed in the fish brain, mainly focusing on spatial memory and avoidance learning as well as social interactions. The neural correlates of most other cognitive functions in fish are still unknown. Two methods used to investigate brain activation patterns in vertebrate groups are looking at immediate early genes and the phosphorylation of ribosomal proteins (pS6). The aim of this project was to investigate the neural substrates involved in visual learning in the fish *Pseudotropheus zebra*. In particular, the expression of pS6 was compared in three different behavioral and learning contexts. All fish were kept in isolation for at least one week. The control group (N=10) was sacrificed with minimal interactions. Fish in the stress group (N=10) were prodded with a net for an hour and then sacrificed. The third group received daily training sessions to associate a visual object with food reward. Fish (N=10) were sacrificed the day they reached the learning criterion. The activation of cells was measured using pS6 as a marker in 20 areas of the telencephalon, diencephalon and mesencephalon. Compared to the control group, both experimental groups showed the largest activation in the inferior lobes (hypothalamus) and some activation in the tectum opticum. The stress group also showed activation in the preoptic area and several telencephalic regions. The activation of the preoptic area is likely associated with the increased stress level, while the activation of the posterior dorsal area may have been caused by olfactory stimulation from the net and the activation of the medial dorsal area by an avoidance learning component, all of which were not present in the training situation encountered by the third group. Activation of the central dorsal area of the telencephalon in the trained group in turn could have been caused by a spatial component of the training situation. Our study showed for the first time that the inferior lobes of the hypothalamus play an important role in visual learning.

Landscape type affects the functional diversity of carabid beetles in agricultural landscapes

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Agricultural intensification and expansion has been suggested to be a major driver of the loss of biodiversity and associated ecosystem services. While on-site management is evidently a significant factor, the importance of landscape-level effects for insect declines may have been previously underestimated. We here compared carabid beetle (Coleoptera, Carabidae) assemblages of wet meadows among three pairs of highly fragmented ‘modern’ and less fragmented ‘traditional’ agricultural landscapes, using pitfall trapping. Taxonomic diversity and abundance of Carabidae did not differ between modern and traditional landscapes, whereas the proportions of brachypterous, large, and habitat specialist species was lower in modern compared to traditional landscapes. These results suggest that landscape type affects functional rather than taxonomic carabid beetle diversity, based on species turnover. To preserve functional diversity in agricultural landscapes, conservation strategies should consider the landscape level in addition to on-site management, by securing connectivity between remnants of semi-natural habitats.

Insight into the olfactory system of the migratory locust, *Locusta migratoria*: An anatomical and cellular study

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In contrast to the stereotypically distinct glomeruli and the uni-glomerular wiring logic of olfactory sensory neurons (OSNs) found in the antennal lobes (ALs) of most insects studied, locusts display a unique AL organisation, with more than 1000 fused and singular micro-glomeruli innervated by multi-glomerular axonal projections of OSNs and dendritic processes of projection neurons (PNs). These features make the locust an interesting case for evolutionary and comparative studies of the insect olfactory system. In our study, we aim to thoroughly characterise the anatomical and cellular architecture of the olfactory system in the migratory locust, *L. migratoria*. Through anterograde staining of sensory neurons, immunohistochemistry and light scanning confocal microscopy, a 3D brain atlas of olfactory-related neuropiles is under construction. This includes in particular the AL, the lobus glomerulatus (LG) and the median crescent (MC). These three neuropiles comprise glomerular structures and receive sensory inputs of either exclusively olfactory or diverse chemosensory neurons housed in antennal and palp sensilla. So far, we successfully traced OSNs of identified antennal sensillum types into the AL, using single sensillum anterograde stainings. These results will shed light on circuitry patterns with respect to sensillum type and topology. Besides the huge number difference of OSNs housed in the two olfactory types of sensilla (basiconica and trichodea) previously described, we observed wiring and morphometric variation. For instance, OSN projections from both types of sensilla arborize onto a similar number of AL synaptic domains, composed of a few adjacent glomeruli. However, basiconic OSNs display denser axon terminal arborisations in these domains compared to trichoid OSNs. In addition, while trichoid OSNs in general appear to have similar morphometric characteristics, basiconic OSNs display a pronounced heterogeneity and can be categorised into different types (e.g., extended and restricted dendritic arbours). Finally, we also investigate the AL wiring of the synaptic counterparts of OSNs, the PNs, by means of focal injections into the mushroom body calyces.

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Neuropeptides in a classical shrimp neurohaemal organ, the postcommissural organs of *Palaemon serratus*

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Nerves and neurohaemal organs emerging from the crustacean tritocerebral commissure, the so-called postcommissural organs (PCOs), were first described in 1953. We have reinvestigated structures and connectivities of the PCOs in the shrimp *Palaemon serratus* by immunocytochemistry in whole mounts and sections, and studied their neuropeptide contents by mass spectrometry (MALDI) and by in-vivo chromatophore bioassay. PCOs contain several neuropeptides in typical neurohaemal endings but no cell bodies of neurons. Most prominent are red-pigment concentrating hormone (RPCH) and corazonin peptides, as identified by MALDI. The origins of RPCH- and corazonin-neurons are traced back to two distinct neurons each in the eyestalk. However, several more masses of as yet unidentified neuropeptides ranging up to 3000 Da have been found in PCO-extracts. Injection of PCO extracts into dark-adapted shrimps with dispersed large red chromatophores showed that the PCOs contain (a) factor(s) that rapidly induce long lasting red chromatophore concentration. First analyses showed that this bioactivity was partially mimicked by synthetic RPCH, but corazonin, known as associated with a similar bioactivity in crayfish, was found to only be active at extremely high concentrations. Previously described nerve fibres running through the PCO to so-called moulting muscles and their possible activation by PCO neuropeptides deserve future studies.

Molecular adaptations in the lung and muscle transcriptomes of parasitic infested harbour porpoises *Phocoena phocoena*

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The harbour porpoise is the only native cetacean in German waters. Anthropogenic activities in the North and Baltic Sea have been increasing over the years, simultaneously negatively affecting the populations of the whales. With dwindling numbers and increasingly declining health multiple monitoring and conservation networks were established. Recently, it has been observed that harbour porpoises use only 60% of their possible lung volume. Additionally, accumulating parasitic and pathogenic damages to the respiratory apparatus of German harbour porpoises were confirmed. It is not known if these factors correlate. To date, few studies have analysed if they impair the diving ability, the swimming speed and ultimately the foraging success. Furthermore, it has been observed that harbour porpoises perform unplanned dives to avoid underwater noise. Damaged lungs could imply a lower oxygen uptake, which in turn hinders the effective oxygen supply to the swimming musculature, resulting in an insufficient diving ability. This project aims to whether harbour porpoises developed molecular adaptions to compensate for this, thus remaining viable and competitive. For this, initial comparative transcriptome sequencing (RNA-Seq) will be used to identify differentially expressed genes in healthy and damaged lung and muscle tissue that may be involved in the compensation of reduced oxygen uptake. Quantitative qRT-PCRs will be applied to verify mRNA gene expression of selected candidate genes in a larger subset of samples. Protein expression will be quantified using Western Blot and ELISA. Finally, the function of the targeted genes and pathways will be investigated in an overexpression- / knock out-assay using the CRISPR/Cas9 system under hypoxia in transfected cell lines. Initial results of the RNA-Seq indicate a differential gene expression between healthy and damaged tissue in both lungs and muscles. Pathways and biological processes such as response to oxygen levels, regeneration, regulation and translational modifications are upregulated in damaged lung and muscle tissues. These results are currently being analysed in more detail.

Social spacing and group behaviour in juvenile and adult weakly electric fish *Mormyrus rume proboscirostris*

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Mormyrus rume proboscirostris, a nocturnally active, weakly electric fish of the family Mormyridae, live in social groups at least during some life stages. Individuals continuously emit and perceive pulse-type electric organ discharges (EOD) to communicate with conspecifics and to facilitate shoal formation and social cohesion. During electrocommunication, group members exchange identity information via the waveform of their EOD, as well as contextual information through the modification of inter-discharge intervals (IDI). Group behaviour in Mormyrids often changes with age; while juveniles are gregarious and live closely together, adult males form territories, which they defend against conspecifics through aggressive interactions. The focus of this project was to compare how the social behaviour and spacing of juvenile and older *Mormyrus rume* differs, and to explore the possible underlying mechanisms driving this change. For this project, groups of juvenile and adult individuals were observed while interacting freely in an experimental tank. Their behaviour and EODs were recorded, and subsequently analysed. For each individual, next neighbour distance (NND), swimming speed, boldness assessed by distance to the tank walls and the number of aggressive behaviours, such as biting, were quantified. In addition, IDIs were measured to assess the electric signalling behaviour of the individuals during their interactions. Based on previous experiments conducted by our group, we anticipate that juveniles practise stronger group cohesion than adults, and that larger individuals maintain larger NNDs than smaller individuals of the same age. Juvenile fish are also expected to be less bold than the adults. Aggressive behaviours might be more common in groups with larger individuals, and older individuals. With these results we will be able to gain more insight into the development and mechanisms of social behaviours and social signalling in *Mormyrus rume*.

Impact of multigenerational inbreeding on kin mating preferences in *Pelvicachromis taeniatus*

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Inbreeding – mating between close relatives – is thought to be selected against since it can cause a decrease in the fitness of offspring due to inbreeding depression. However, recent theoretical and empirical studies suggest that the advantages of kin mating can override the effects of inbreeding depression and an optimum level of relatedness for both males and females should emerge that can increase fitness. Here, we examined the effect of relatedness on mating behavior in inbred and outbred individuals of the biparental cichlid fish *Pelvicachromis taeniatus*. In previous studies, *P. taeniatus* has been found to express active inbreeding (kin mating preference) with no evidence for inbreeding depression. Parental cooperation was improved in related pairs, potentially rendering inbreeding an adaptive strategy. However, kin mating preference is not unconditional, but at least partly quality-dependent, i.e., high-quality individuals show stronger kin-mating preferences, and low-quality kin are rejected as mates. Furthermore, the costs of inbreeding may change with persistent inbreeding and kin mating preferences may change accordingly. Here, we compared kin mating preferences in fourth-generation inbred (resulting from full-sibling matings) and outbred fish to examine if kin mating preferences might change with progressing inbreeding. A comprehensive mating experiment was conducted in which one male was presented with two females, one unfamiliar kin and one unfamiliar non-kin. We found fish from inbred and outbred lineages to differ in kin mating patterns. Inbred lineages paired more often with unfamiliar non-kin, unrelated females tended to court the male more often than related females, and males courted the unrelated female more often than the related females. In outbred fish, mating tended to occur more often among relatives. While the outbred lineages seem to prefer inbreeding like in previous generations of *Pelvicachromis taeniatus*, preference in the inbred fish was altered, suggesting that the cost-benefit ratio of mating with kin may change with increasing degree of inbreeding.

Body-to-limb proportions and exploratory limb function in stick insects

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Overall body proportions and relative limb length are highly characteristic for many insect species. In case of the legs, limb length has mostly been discussed with regard to parameters of locomotor performance and, in particular cases, as an adaptation to environmental factors or to the mating system. In addition to the walking legs, the present study includes the antennae, i.e., sensory limbs of the head. In particular, we compare three species of stick and leaf insects (Phasmatodea) that differ strongly in the length ratio between antennae and walking legs, with the antennae of *Medauroidea extradentata* being much shorter than its legs, nearly equal length of antennae and legs in *Carausius morosus*, and considerably longer antennae than front legs in *Aretaon asperrimus*. By comparing spatial action ranges of antennae and legs during walking and searching, we show that that relative limb length is directly related to the near-range exploration effort, with complementary function of the antennae and front legs irrespective of their length ratio. Distinctly different shapes and overlap zones of the antennal searching volumes of *C. morosus* and *A. asperrimus* likely reflect species differences in active exploration effort and, therefore, spatial attention. Since *M. extradentata* shows sustained, active antennal movement during locomotion, despite the fact that its antennae are too short for tactile sensing, antennal movement in stick insects must also serve sensory modalities other than touch. Assuming that inter-species differences in limb hold for both sexes and all developmental stages, this study further explores how relative limb length differs between sexes and how it changes throughout postembryonic development. We show that the pattern of limb-to-body proportions is species-characteristic despite sexual dimorphism, and find that the change in sexual dimorphism is strongest during the last two moults. Finally, we show that antennal growth rate is consistently higher than that of front legs, but differs categorically between the species investigated. Whereas antennal growth rate is constant in *Carausius*, the antennae grow exponentially in *Medauroidea* and with a sudden boost during the last moult in *Aretaon*.

Spider vibes revisited: Is male *Pisaura mirabilis* vibratory performance during courtship reliable?

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Arachnids are especially sensitive to vibrations of all types, this is also true for cursorial spiders which perceive vibrations through diverse environmental substrates rather than a stationary web. One such species is the Nursery Web Spider, *Pisaura mirabilis*. This species employs several reproductive tactics which make it especially valuable for study. It is one of only a handful of taxa in which males offer females a nuptial gift during the reproductive process, typically consisting of a prey item wrapped in silk. The male's courtship also includes visual, chemical, and most notably, vibrational stimuli. Courtship vibrations consist of repeated pulses, and advertise the male's physical condition (Eberhard et al., 2020). Our project explores the functional role of vibratory communication within the framework of *P. mirabilis* reproduction. We evaluated the vibratory performance of 150 male spiders by collecting repeated recordings (N=3) and analyzing the pulse train associated with the courtship. Based on temporal variables shown to influence female choice in *P. mirabilis*, we assessed the intra- and interindividual variability of vibrational performance within the sample and categorized males as either "high-signaling" or "low-signaling." Female spiders were then mated sequentially with males of both good and poor courtship performance, and their behaviors indicating preference (such as latency to mate, copulation duration, and sexual cannibalism) were recorded. These were assessed to determine the effect of male vibratory performance on female choice. The newly-developed methods and results from this study will inform future work on the full reproductive consequences (paternity of mixed broods) of male vibratory performance in *P. mirabilis*, as well as the role of vibration within the multimodal courtship signaling behavior of these spiders.

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The global repertoire of taxonomically restricted genes in *Hydra*

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Taxonomically restricted genes (TRGs) are genes that can only be found in specific lineages of organisms, but not in its ancestors. In principle, the term can be applied to genes at any taxonomic level, but is commonly used for “young” genes, restricted to a particular phylum or smaller taxonomic ranks and, therefore, likely to have evolved more recently. Such genes have been reported in all genomes sequenced to-date and they have been shown to be important for adaptation of organisms to novel environments, and for the evolution of novelty, for example in the case of the Cnidaria-specific cell type: nematocytes. Furthermore, preliminary work done in our group has suggested that TRGs could also be important in the evolution of the nervous system in the freshwater cnidarian *Hydra vulgaris* AEP, where taxonomically restricted gene families are enriched in neuronal cell types and precursors. It is evident that the TRG repertoire in cnidarians could provide meaningful insights into the evolution of novelty, but no general study has been done until now. *Hydra*, is particularly suited for the study of TRGs because up to 50% of its genes seem to be taxonomically restricted. In order to address this, we began by combining long-read and next generation sequencing to assemble a high-quality genome of *Hydra vulgaris* AEP. Using a phylostratigraphy approach, we assign an age to each gene with evidence of transcription in the genome, and determine the set of TRGs at the level of phylum (Cnidaria), class (Hydrozoa), family (Hydridae), and species. Furthermore, using publicly available single-cell RNAseq data from *Hydra*, we corroborate the enrichment of taxonomically restricted genes in specific cell types including neuronal cells and precursors. The bioinformatics pipeline that we establish will allow us to study the importance of TRGs for different environmental conditions and developmental stages. Finally, through implementation of diverse bioinformatic tools we attempt to classify the genes according to putative function, cellular localisation, and domain composition. The tools and findings generated through this project can inform future hypothesis-driven experimental work to study the evolution of tissues in *Hydra*, and even other organisms.

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Molecular mechanisms of photoperiod induced sexual reproduction in *Daphnia magna*: a candidate gene approach

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Small freshwater crustaceans of the genus *Daphnia* occur in many freshwater bodies worldwide. As an important part of freshwater ecosystems, *Daphnia* is sensitive to environmental conditions. Under favorable conditions, *Daphnia* females reproduce parthenogenetically. When environmental conditions become unfavorable, *Daphnia* females switch from parthenogenesis to sexual reproduction. The sexually produced haploid eggs will be fertilized by *Daphnia* males that are produced parthenogenetically. The diploid embryos are destined to enter a state of suspended animation termed diapause, by which *Daphnia* population can overcome harsh environmental conditions. The onset of sexual reproduction, i.e., production of diapausing embryos, is indicated through distinct environmental factors, i.e., crowding in combination with changes in the photoperiodic length. A previous study revealed that G protein-coupled receptor rhodopsin, a photoreceptor gene, is involved in diapause induction in *D. magna*. This finding provides a mechanistic explanation for the link between diapause and day-length perception. To test this hypothesis we established a solid bioassay in which *D. magna* females were cultivated under a long (16:8 h light: dark cycle) and a short (8:16 dark: light cycle) photoperiod, the number of male offspring as well as the number of sexually produced embryos were determined. With this, we will now perform RNAi and CRISPR/Cas to knock down/out this target rhodopsin. We will then determine the production of male offspring and ephippia. The present study aims to obtain a better understanding of the molecular mechanisms responsible for the regulation of sexual reproduction in *Daphnia*.

Influence of inbreeding on shoaling behaviour in juvenile cichlid fishes

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Living in shoals is a common behaviour in fishes, due to beneficial effects, like protection against predators and improved success in foraging. It has been shown in several fish species that individuals can discriminate between kin and non-kin conspecifics and that they prefer shoaling with kin. Shoals in natural populations have varying degrees of inbreeding, which on one hand can have disadvantages because of inbreeding depression, but in the other hand it can increase the fitness in optimal adapted individuals. However, knowledge on the influence of inbreeding on kin recognition and shoaling behaviour is scarce. In addition, shoaling preferences in the presence of other conspecifics has been rarely addressed. Here, we investigated the effects of inbreeding on shoaling preferences in juveniles of *Pelvicachromis pulcher*. In the experiment, we gave inbred or outbred fish the choice between familiar kin and unfamiliar non-kin shoals, consisting of 4 conspecifics each and analysed the shoal-preference, activity and sociality. Furthermore, we compared the shoaling behaviour of single fish and fish in dyads. We expect a preference for kin due to indirect fitness benefits. Higher degree of relatedness in inbred fish could lead to stronger preference for kin in inbred fish. In contrast, potential reduced cognitive abilities of inbred fish due inbreeding depression might negatively affect kin discrimination and shoaling behaviour. For the dyads we expect a higher activity and lower sociality level than for the single fish.

Cellular mechanisms of predator induced defence development in the freshwater crustacean *Daphnia lumholtzi*

Natascha Sophia Gaedigk; Annette Graeve; Linda C. Weiss

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Freshwater crustaceans of the genus *Daphnia* are well-known to express morphological defenses in the presence of predators. These morphological defenses can show in different forms ranging from helmets, neckteeth, and crests to elongated head spines. These features grow upon the exposure to predator specific chemical cues, so-called kairomones and reduce the predation risk through posing handling difficulties on the predator. While research has mostly centered on the ecology and evolution of *Daphnia* defenses, there is only limited information on the cellular mechanisms that underlie site specific tissue growth. Distinct polyploid cells have been described, that were discussed to control site specific cell proliferation. Time dependent cell proliferation patterns that underly defense development have up to now only been described in *D. longicephala*. Here, cell proliferation first occurs in forms of increased cellular growth, which is not accompanied by an increase in mitotic activity until 72 h post predator exposure. However, as these extensive crests developed by *D. longicephala* are morphologically different from the elongated head spines in *D. lumholtzi*, we will here determine cell proliferation patterns in *D. lumholtzi* exposed to fish kairomones. With the help of a cell proliferation assay (Click-iT™ EdU Cell Proliferation assay) we monitor changes in the epidermal tissue of naïve and predator exposed *D. lumholtzi* over a time series of 72 hours. The degree of mitotic activity will be elucidated with the help of a mitosis marker (phospho-histone 3 (Ser10)). Our results will provide new and comparative insights into the cellular processes underlying morphological defense expression in different *Daphnia* species.

A look into early pterosaurian feeding ecology by quantifying head and tooth shape

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Pterosauria is an ingroup of Reptilia; its representatives are known from the late Triassic until the end of the Cretaceous. They are also the first representatives of Vertebrata that have evolved powered flight. This novel way of locomotion also opened up new feeding opportunities. Adding somewhat free vertical movement allowed for feeding in spaces that were otherwise inaccessible for other animals at the time. Most research into pterosaurian feeding ecology has been conducted via simple distance morphometrics or dental microwear analysis. While such analyses often come to interesting conclusions, most of them work with only a few individual features of the skull or jaw. Here we aimed at exploring the skull and tooth morphospaces of early pterosaurians via quantitative shape analysis. This was done via Elliptic Fourier Analysis, which was used to quantify the outline of the object in question. Using a combination of lateral view images from collection visits and from the established literature a dataset of skull drawings was fashioned and analysed using the program Shape. The tooth shape spans from elongated and curved teeth to shorter more triangular ones. Teeth of animals that are suspected “piscivores” seem especially distinct. Some representatives of Archaeopterodactyloidea show differences between immature and adult specimens. The skull shape reaches from stout and more triangular skulls to elongated flatter skulls. Skulls from immature representatives of Archaeopterodactyloidea are different for some ingroups: early branching representatives show very different shapes between immatures and adults of the same species. These differences become smaller throughout time, as later representatives of the group showed fewer differences between developmental stages. Head shapes of immatures of early branchings of Archaeopterodactyloidea and early relatives of modern birds share some similarities. These findings seem congruent with the idea that the emergence of birds during the Jurassic lead to ecological competition between the former and pterosaurians. The results indicate that some early branching Archaeopterodactyloidea had some form of niche differentiation between stages. The change in immature skull shape and dentition throughout the group's history could have been influenced by birds that competed for the same food as the immatures.

postpb – intuitive graphical assessment of Bayesian phylogenetic analysis outcomes

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Bayesian phylogenetics using Markov chain Monte Carlo (MCMC) has become one of the most popular and widely used approaches to reconstruct evolutionary relationships. Several software implementations allow Bayesian phylogenetic reconstructions based on a variety of data types and a multitude of evolutionary models. The MCMC approach produces samples of model parameters and trees, which need to be evaluated and summarised in order to determine the outcome of an analysis and ultimately, the phylogeny of interest. Such posterior analyses are crucial in assessing robustness of phylogenetic estimates, yet are rarely reported to sufficient detail. Probing 200 recently published studies employing the tool “Phylobayes” revealed that a majority reported the model and number of MCMC chains employed. However, only about 50% of studies specified the number of generations used for sampling or topology differences between chains, and even fewer (< 25%) mentioned effective sampling sizes for model parameters. This inadequate reporting on posterior analyses hinders interpretation and reproducibility of phylogenetic studies. The issue may arise from an absence of broadly agreed minimal reporting standards for Bayesian phylogenetic analyses, and also from the dearth of accessible tools enabling such reporting. I here present postpb, a computational tool for posterior assessment of Bayesian phylogenetic analyses, e.g., from Phylobayes or Mr. Bayes. Postpb can be used to graphically display trace files and calculate various summary statistics that help in assessing convergence and mixing of chains, and to determine appropriate burnin sizes. It includes a consensus tree viewer that supports interactive editing and different approaches for exploring phylogenetic conflict between tree samples from different chains. To facilitate reporting and integration into phylogenetic workflows, all graphs and statistics produced in postpb can be exported. Postpb is an interactive and easy to use Shiny app that is platform independent and requires no prior knowledge of the R or Shiny environments. Postpb thus facilitates standardised reporting on, and reproduction of Bayesian phylogenetics and makes posterior analyses more accessible.

Early olfactory processing in antennal lobe neurons in the stick insect *Carausius morosus*

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For reliable spatial orientation in darkness, where the use of vision is limited, many insects rely on other sensory modalities such as touch, gustation, or olfaction. Insect antennae, being dedicated multi-sensory organs, play a key role in the sensation of these modalities. The stick insect *Carausius morosus* carries two long antennae and several studies have addressed their use in active tactile sensing and the sensory control of locomotion. During walking, the antennae are constantly moving in a rhythmic manner and show an increased sampling frequency upon antennal contact events that guide targeted leg movements. So far, olfaction has been studied only very little in stick insects. However, since their antennae carry many chemosensory sensilla, antennal sampling is likely to provide chemosensory information in addition to tactile and proprioceptive information. As a first step towards multimodal integration of antennal sensory information, the present study focuses on olfactory processing at two stages: the peripheral sensory activity (via EAG) and the first processing stage (via multi-unit recordings of antennal lobe (AL) neurons). A key objective was to test different odour components for differences in relative activity induced at the periphery and at the AL. Early results show that the odour-specific activity at the AL processing level differs from the odour-specific signal strength at the peripheral receptor level. In *C. morosus*, we could show this for the odour components hexanal and citral. Since hexanal is a characteristic odour component of freshly cut leaves and citral, a mixture of the cis-trans isomers geranial and neral, is responsible for a fruity smell, both odour components are likely to be of behavioural relevance to herbivorous stick insects. Follow-up studies will need to test whether the prominent separation of hexanal and citral in AL activity is mirrored by behavioural sensitivity or choice preference. Methodologically, our results lay the basis for future investigation of cross-modal integration in antenna-mediated behaviour.

Elevated pCO₂ affects neuronal plasticity underlying predator perception in the freshwater crustacean *Daphnia longicephala*

Deria Maryan Görl; Linda Weiss

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With industrialization atmospheric CO₂ increased substantially during the past decades. This has been shown to have significant effects on different ecosystems. For example, increased atmospheric CO₂ dissolves in aquatic habitats and has a negative impact on olfactory and visual senses thereby changing species interactions. In some cases, elevated levels of pCO₂ thereby reduce predator induced defense expression, as in the case of the water flea *Daphnia*. *Daphnia* is thereby less defended and more prone to predation. However, the effect of pCO₂ on the neuronal and cellular mechanisms involved in defense expression are not understood. We studied this in the freshwater crustacean *Daphnia longicephala*. When exposed to predators, *D. longicephala* develop defensive crests, which are associated with neuronal plasticity, where the brain volume increases together with a rewiring of the inhibitory neuronal connections. When exposed to elevated levels of pCO₂ this neuronal plasticity is impaired, so that there is no brain volume increase, and we find a reduced number of inhibitory neuronal connections in comparison to the ambient treatment. Our results demonstrate the first functional impact of how elevated pCO₂ levels affect species interactions on the neuronal level.

From wasps' eyes this thesis we derive - Comparative morphology and phylogenetic significance of modified ocelli in digger wasps.

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Most flying insects possess not only two compound eyes, but also three additional single-lens eyespots on the vertex. These ocelli form a secondary visual system, which was long believed to function as a simple light intensity sensor. However, recent studies have demonstrated that ocelli are more complex than previously thought, enhancing not only altitude, roll and pitch during flight, but also navigation by detecting patterns of celestial polarisation. The morphology of ocelli is highly specialised and correlates with specific lifestyles. Their most prominent features are a bipartite retina and, in Hymenoptera, the rhabdomeric organisation into "hymenopteran plates". Among the paraphyletic digger wasps, the ocelli are modified, reduced, or the posterior ocelli completely lost. These heteromorphic reductions among several non-sister clades are likely caused by a functional convergence of the homologous lens, iris and retina, called a homoiology. While unique ocellar modifications are a commonly used trait in pre-genetic digger wasp taxonomy, their internal morphology is completely unknown. Why have reduced ocelli evolved multiple times independently within digger wasps, while such modifications are otherwise unknown in insects? Do these reductions lead to a loss of function, or represent a gain of new function leading to adaptation? What phylogenetic conclusions for this enigmatic paraphylum can be drawn from these traits? To answer these questions, we will apply a combined approach of μ CT scans, light and electron microscopy. We hope to improve this technique, used in multiple publications, by conducting all techniques on each specimen, enhancing comparability between specimens. Further, we want to expand the analysis of light and electron microscopy pictures by morphometric measurements. To this end, we will conduct a principal component analysis of landmarks placed in the 3D models obtained from the scans. This allows us to investigate differences of modified to unmodified ocelli as well as differences between species with modified ocelli.

The Tracheal dendrite neurons of *Drosophila* are sufficient to trigger eclosion behavior but are not required for its rhythmicity

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In order to grow, arthropods must shed their exoskeleton and replace it with a new one through a process called molt. Each molt ends with ecdysis, a stereotypic and peptide-orchestrated motor pattern that serves to shed the old cuticle. The final ecdysis that leads to the emergence of adults from the puparium is circadianly gated to the morning hours in *Drosophila*. During each molt, a sequence of events including tracheal collapse, ecdysis motor program, and finally airway clearance happens. During the tracheal collapse, the space between the new trachea lining and the old cuticle is filled with molting fluid and causes temporary hypoxia. Two types of specialized gas-sensing neurons are present in the vicinity of the trachea. These peripheral ascending neurons are the ventral Tracheal dendrite 1 and 2 (v'Td1 and v'Td2) and are important for O₂ and CO₂ sensing respectively. In the ventral nerve cord, these neurons pass close to the descending projections of the Ventromedial (Vm) neurons, a pair of large secretory neurons releasing Eclosion Hormone (EH). Using a set of connectivity tracing techniques, we found that v'Td neurons are presynaptic to the Vm neurons. These synaptic connections seem functional as activation v'Td neurons result in Ca²⁺ activity in the Vm neurons. Moreover, optogenetic activation of v'Td neurons is sufficient to initiate premature eclosion. Yet, conditional ablation of the v'Td neurons did neither affect the eclosion nor its rhythmicity. As the peptide Leukokinin (Lk) is required for airway clearance, we tested whether Lk signaling is required for eclosion rhythmicity. However, mutations in Lk or the Lk receptor did not affect eclosion rhythmicity. Unlike v'Td neurons, we could not induce premature eclosion by optogenetically activating the Lk neurons.

Information integration for nutritional decision-making in desert locusts

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Swarms of the migratory desert locust can extend over several hundred square kilometres, and starvation compels this ancient pest to devour everything in its path. Theory suggests that gregarious behaviour benefits foraging efficiency over a wide range of spatial food distributions. However, despite the importance of identifying the processes by which swarms locate and select feeding sites to predict their progression, the role of social cohesion during foraging remains elusive. We investigated the evidence accumulation and information integration processes that underlie locusts' nutritional decision-making by employing a Bayesian formalism on high-resolution tracking data from foraging locusts. We tested individual gregarious animals and groups of different sizes in a 2-choice behavioural assay in which food patch qualities were either different or similar. We then predicted the decisions of individual locusts based on personally acquired and socially derived evidence by disentangling the relative contributions of each information class. Our study suggests that locusts balance incongruent evidence but reinforce congruent ones, resulting in more confident assessments when evidence aligns. We provide new insights into the interplay between personal experience and social context in locust foraging decisions which constitute a powerful empirical system to study local individual decisions and their consequent collective dynamics.

SynGAP function and regulation of Ras signalling cascade

Taanisha Gupta

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The brain is the center of the nervous system and a complex organ that controls every process that regulates the body. Neuronal cells process and transmit information via electric or chemical signals via neuronal junctions known as synapses. Excitatory glutaminergic synapses exhibit a prominent postsynaptic density (PSD), a network of numerous kinds of proteins such as kinases, scaffold proteins, and GTPase activating proteins (GAPs) (Dosemeci, Weinberg et al. 2016). One such GAP is SynGAP. SynGAP Syndrome is a rare genetic, neurodevelopmental disorder (NDD) caused by mutations in the SYNGAP1 gene (Hamdan, Gauthier et al. 2009). Loss-of-function mutations in the SYNGAP1 gene cause protein truncation resulting in SynGAP haploinsufficiency (Melom and Littleton 2011). With an incidence of 6 per 100,000 individuals, SYNGAP1 variants are astonishingly common, however, it is often underdiagnosed and only ~1055 cases are known as of 2022. SynGAP was initially recognized as a Ras-specific GTPase activating protein (RasGAP). SynGAP1 acts upon small GTPases such as Ras and Rap. Provided that SynGAP1 variants are one of the prevalent causes of ID, exploring the causes of the syndrome is of relevance. We aimed to develop a relevant cell-based assay to assess the activity of RasGAP (SynGAP protein) and portray how it regulates the Ras pathway through analysis of protein phosphorylation using HEK cells. We developed an assay by overexpressing SynGAP to understand its role and establish a working model for future therapeutic targets. In doing so, we went through various optimization techniques involving the application of various methods such as western blotting, immunofluorescence, and in-cell westerns and using overexpressing SynGAP interaction partners such as scaffold proteins (PSD95), Calcium, and CDK5. To analyze the protein expression, we used ImageJ and GraphPad Prism6. We were successfully able to activate the Ras-Erk pathway in our hands. Preliminary results with SynGAP overexpression show weak basal GAP activity validating its role as a RasGAP. Interestingly, results also display colocalization of SynGAP and NMDAR regardless of PSD95 indicating a direct interaction between SynGAP and NMDAR. With further optimization and translating this assay to neuronal cells can offer larger GAP activity of SynGAP, providing more insights into the biochemistry.

Neuronal organization of a layered structure in the dorsal forebrain of gobies

Ruth Gutjahr; Boris Philippe Chagnaud

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Goby fish exhibit a remarkable level of organization in the caudo-dorsal region of the telencephalon, where small, granule cell-like neurons are stacked into distinct layers. This extent of organization has not yet been reported in the forebrain of other fish families. However, in different species, a layered organization of neurons is known to facilitate parallel and reciprocal processing in neuronal networks, suggesting advanced input processing. We performed tracing experiments, using in-vitro dye electroporations and pin-injections to assess the connectivity of this layered structure with different nuclei in other brain areas. These tracing experiments revealed fibers extending to other divisions of the telencephalon, as well as to two midbrain nuclei. Additionally, we investigated the morphology of single neurons within different layers using single-cell Dil-electroporations in fixed forebrain slices. We found that neurons are characterized by a limited dendritic arborization and are densely packed with dendritic spines, which resembles the morphology of the granule cells of the cerebellum and the hippocampal dentate gyrus. Using immunohistochemistry, we found that a number of neurons within the layered structure receive Substance-P and parvalbumin-immunoreactive fibers and express GLS-2. Our findings indicate that this layered neuronal structure is a part of the dorsolateral area of the telencephalon (DI), which is considered to be homologous to the medial pallium, including the hippocampus in mammals. In the future, we will use electrophysiological experiments and calcium imaging experiments to further investigate the functionality of the neurons within these layers.

Go with the flow: Passive flow in two benthic suspension feeders

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Suspension feeders are a group of organisms with the common ability to separate food particles from the surrounding water. Their feeding activity influences particle fluxes, moves high volumes of water, and even forms habitats. While passive suspension feeders use the ambient current to feed, active ones produce a current through ciliary activity, pumping, or forward swimming. Some active SFs might use certain aspects of their morphology to draw a passive current through the separation medium, possibly to save energy. In this context, we analysed the fluid-shape interactions in a tunicate and a cephalochordate using 2D morphometrics and computational fluid dynamics to determine the effects of changing flow velocity, open area of the gill slits in the branchial basket, feeding orientation, length of the organism, siphon/mouth geometry, depth in the sediment, and surface conditions in a benthic environment. Due to the differences in size and diameters, the Reynolds number was 0.25 at the mesh with a volume flow of $450 \text{ mm}^3/\text{s}$ in the tunicate and $Re = 0.07$ and $2.7 \text{ mm}^3/\text{s}$ in the cephalochordate at an ambient flow velocity of 0.3 m/s indicating laminar flow. The volume flow generally increased with the increasing ambient flow velocity. When the tunicate was tilted towards the flow direction, the volume flow from inhalant to exhalant siphon flow increased to around $550 \text{ mm}^3/\text{s}$, corresponding to 2 l/h . Similar to the ascidian, the orientation of the cephalochordate within the flow had the highest impact on passive volume flow relative to the other parametric changes. Our results are a first indication that the morphology of both active suspension feeders can induce a passive current through the separation medium, but volume flow and direction are influenced by the orientation of the organisms and ambient flow conditions.

Does an enriched environment impact the synaptic plasticity in the hemiellipsoid body of the marbled crayfish?

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The pathogenetic Marbled Crayfish *Procambarus virginalis* produces genetically identical offspring, making it a unique and ideal study organism in neuroscience. For investigating how and on which level environmental factors affect neuroplasticity in this emerging model organism, we used 35 siblings of one batch in two different rearing regimes. One was impoverished (kept separately without any social contact, small and sparsely furnished aquariums, constant diet and feeding times), the other was enriched (kept in group of siblings in a large aquarium, varying the diet and feeding times, rearrangement of hiding places and water flow). Four time points (the day of separation, after 3, 6, and 12 weeks) were determined at which individuals of each rearing regime underwent the same procedure: 1. Measurement of the carapace from the tip of the rostrum to the posterior edge, 2. µCT-scanning of the head, 3. histological sectioning followed by 4. Immunohistochemical labeling of GAD (glutamic acid decarboxylase, a precursor of the inhibitory neurotransmitter GABA) and synapsin as well as a nuclear counterstaining. By using the carapace lengths and the 3D-reconstructions from µCT-scanning as references controlling for allometric growth, we will compare the changes in the nervous system of individuals of both rearing regimes. Here, our main focus is on the hemiellipsoid body of the lateral protocerebrum, a higher-order neuropil which is presumed to be responsible for learning and memory. In addition, we will compare the synaptic densities in the hemiellipsoid body of both groups as revealed by immunohistochemical labeling of synapsin (presynaptic contacts) and GAD (postsynaptic contacts of mainly inhibitory neurons). We hypothesize that individuals which were reared in the enriched environment and thus experienced more sensory input, will possess larger hemiellipsoid bodies and higher synaptic density in these putative centers for learning and memory.

Understanding variability in nematode development on single-embryo and single-cell level

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The phylum Nematoda consists of a huge diversity of species that are either free-living in terrestrial or aquatic habitats or parasitise animals or plants. Despite this variety of lifestyles, adult nematodes share a universal vermiform Bauplan. However, their embryonic development, especially in the early stages, is highly variable. Morphological differences concerning cell polarisation, cleavage patterns, cellular arrangements, and cell lineages have been studied in many species. Only few nematode species, such as the model organism *Caenorhabditis elegans*, have been investigated in detail on the level of individual gene functions. Prior studies have shown that important developmental genes and pathways in *C. elegans* and nematode species with different phylogenetic distances to *C. elegans* vary in their expression patterns, as well as in their presence and absence in the genome. We performed phylogenetic analysis on ortholog clusters of proteins involved in cell polarisation in *C. elegans* to investigate their conservation in other nematode species. Proteins involved in the polarisation process itself are conserved for the most part, but regulators important for this process in *C. elegans*, are missing in many species. We are aiming to investigate nematode species from all clades to better understand the evolution of genetic pathways in nematode development. We are using the Cel-Seq2 single-cell RNA-Seq method to compare gene expression on single-embryo level throughout development and are currently creating such time course datasets for *Diploscapter coronatus*, a species in the same clade as *C. elegans*, as well as *Plectus sambesii*, a more distantly related species. We are also currently adapting methods to separate single cells for a selection of embryonic stages in different nematode species in order to apply single-cell RNA-Seq. Comprehensive studies including species of more clades will identify the range of possible variability and key pathways with a high degree of conservation.

Detection and regulation patterns of the transcription factor FoxO in diapausing and non-diapausing pupae of *Pieris napi*

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Diapause is a form of deep dormancy that many organisms use in order to survive unfavorable environmental conditions. Butterflies like *Pieris napi* use this adaption to survive harsh winter conditions typical of the temperate zone. The diapause in *P. napi* occurs in the pupal stage and consists of three different stages: induction, maintenance and termination. During these phases *P. napi* first develops the diapause phenotype (induction), then shows complete developmental arrest (maintenance) after which diapause is terminated (upon receiving correct environmental signals) and development, i.e. metamorphosis, can proceed. The molecular processes regulating these complex physiological reconstructions remain largely unknown, and here we present novel data on one putative mechanism, FoxO (forkhead transcription factor). This transcription factor regulates developmental and stress-related responses, including diapause, in other insect species and could be a key regulator acting as major developmental switch in diapause also in *P. napi*. As a transcription factor, FoxO binds to DNA and activates gene expression. The main regulation of FoxO is via the PI3K/AKT-pathway. We studied FoxO and Akt in diapausing and non-diapausing pupae of *P. napi* at central time-points during respective developmental trajectory in order to establish whether this pathway is active/inactive during fundamental developmental transitions, including the stages from induction to maintenance and finally to post-termination of diapause. For this, we used semi-dry Western blot analysis of soluble proteins extracted from brain and abdomen tissue of pupae and detected proteins (FoxO and Akt) via matching antibodies. In addition, we investigated whether temperature manipulation has an impact on FoxO expression dynamics, because termination of diapause is dependent of cold temperature.

Functional assessment of putative chemo-coreceptors IR25a and IR93a of predator perception in *Daphnia*

Joshua Huster; Annette Graeve; Linda Weiss

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The freshwater crustacean *Daphnia* is well known to adapt to an increased predation risk, through the expression of predator specific inducible defenses. Some of these defensive strategies can be seen in form of morphological adaptations, like the formation of spines, thorns or neckteeth, increasing the individual's fitness. The expression of these inducible defenses are initiated by predator specific chemical cues. However, the mechanisms of chemoreception in *Daphnia* have only been studied to a limited extent and especially the involved chemoreceptors remain unknown. Ionotropic receptors (IRs) are discussed as a central element of chemoreception in crustaceans. *In silico* two putative co-receptors have been suggested, i.e. IR25a and IR93a, that together with dedicated tuning receptors form a functional chemoreceptor. We here tested the involvement of IR25a and IR93a in predator perception in three *Daphnia* species (*D. magna*, *D. longicephala* and *D. lumholtzi*) responding to three different predator cues (*Triops*, *Notonecta*, and *Gasterosteus*). With the help of RNA- interference (RNAi) we knocked down IR25a and IR93a. Knock down animals did not express defensive features indicating that the animals lose their sense of smell. We confirmed knock-down of the target gene using quantitative PCR. This study provides a first functional proof of both chemo co-receptors being involved in predator perception and probably chemo perception per se.

Light Trails of Flashlight Fish

Peter Jägers; Timo Frischmuth; Stefan Herlitze

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The flashlight fish *Anomalops katoptron* can be found during dark nights at the water surface in the Indo-Pacific. Aggregations range from small to large and unordered to highly polarized groups. *A. katoptron* are characterized by bioluminescent blink patterns of sub-ocular light organs densely packed with bioluminescent, symbiotic bacteria. Light organs are important for shoaling under dim light conditions and control nearest neighbor distance. Distraction of predators is widespread in the animal kingdom and substantially increases species fitness. In the related flashlight fish species *Photoblepharon steinitzi* confusion of predators via “blink and run” pattern has been proposed. We recorded three-dimensional swimming profiles of *A. katoptron* and correlated them with blink patterns under laboratory settings. Immediately after *A. katoptron* switched from light organ exposure to occlusion, a change in movement direction in combination with accelerated swimming speeds was observed. Swimming speeds peaked during light organ occlusion. Before exposing the light organs again, a continuous decrease in swimming speed was recorded. The phase transition from light organ occlusion to exposure was not followed by a significant change in movement direction. Lowest swimming speeds were detected while light organs were exposed. Solitary individuals showed increased swimming speeds and higher angles during directional changes compared to small groups of *A. katoptron*. Our results indicate that *A. katoptron* combine acceleration with a directional change during light organ occlusion. We explain this behavior with the distraction of predators during schoaling.

Morphology and sky compass signaling of central-complex neurons in the brain of the Madeira cockroach

Stefanie Jahn; Vanessa Althaus; Naomi Takahashi; Juliana Schott; Mona Janning; Uwe Homberg

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Navigation is an essential ability for animals to find food or to migrate to different areas. Celestial cues like the position of the sun or the polarization pattern of the sky are used by many insects to navigate in diverse environments. In the insect brain, the central complex (CX), composed of the protocerebral bridge (PB), the upper (CBU) and lower division of the central body (CBL) and a pair of globular-shaped noduli, plays a major role in navigational tasks and motor control as shown for cockroaches by extracellular recordings in tethered and freely moving animals (Varga et al. 2017, *Front Behav Neurosci* 11:4). To uncover the cellular basis of these data, we studied the neuroarchitecture of the CX through dye-filled neurons and immunostainings. In addition, we used intracellular recordings from tethered cockroaches to study the responses of CX neurons to sky compass signals such as polarized light and stimuli mimicking solar azimuth. The neuroarchitecture and composition of neuronal cell types of the cockroach CX differ from those of the CX in other species in several aspects. The CBL, rather than subdivided into layers, shows an organization into arrays of intersected cones (8) and teeth (9). Many types of CX neurons have prominent ramifications in a large anterior lip neuropil, not present in holometabolous species, and certain types of columnar neurons connecting the CBU and lateral accessory lobe have additional ramifications in the CBL. Recordings from five types of CX neurons that are homologous to cell types in other species showed sensitivity to sky compass signals, such as zenithal polarization or simulated solar azimuth, but in other recordings the same cell types were insensitive to these celestial cues. The data, therefore, show stronger state dependent suppression of sensitivity to sky compass signals than in other species and reveal anatomical specialties that might be related to a nocturnal lifestyle.

Developmental changes in the head of the dragonfly *Libellula depressa* (Odonata: Libellulidae) revealed by geometric morphometrics

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Most animals undergo radical morphological changes during their development. For example, hemimetabolous insects show a comparatively quick embryonic development from egg to subadult but the subsequent subadult stages mainly grow in size towards the adult stage. The allometric relationships between different organs are however unclear because quantitative studies are scarce. Here we present a comprehensive geometric morphometric dataset of the head of the dragonfly *Libellula depressa* (Anisoptera: Libellulidae), compiled through a long-term rearing project. Ten specimens each of 12 larval instars, and the adult stage, were scanned using synchrotron-radiation micro-computed tomography ($n=130$) to landmark homologous loci on the head capsule and the mouthparts. The resulting landmark-set was then used to carry out analyses of allometry and modularity. The differences in shape were computed and visualized using a principal component analysis and three-dimensional surface models. Furthermore, the dataset can be used to examine shape changes in the context of ecological variables, e.g. a changing food spectrum during ontogenesis. Our data allows to quantify the allometric relationships and the modular development within larval stages of this dragonfly and therefore is a starting point to further our understanding of insect head development.

Castration vs. Vasectomy in male guinea pigs - consequences for behaviour and cortisol reactivity

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Animals in human hands should not reproduce uncontrollably. In order to achieve this goal, castrations, i.e. a complete removal of the gonads, are usually performed. This procedure can also lead to a reduction in undesirable aggressive as well as courting and sexual behaviour. These changes in behaviour are in the interest of humans. From the animal's point of view, however, the question arises whether sterilisation would not be better than castration. This procedure is much less invasive, as the gonads are not removed, but only the transport of the egg or sperm cells is prevented. The overall objective of this study was to analyse the different effects of castration and sterilisation on animal welfare, using the domestic guinea pig as an animal model. To achieve this goal, we studied four adult vasectomised and six adult castrated males (age of surgery: 30-40 days). Each male was housed together with two females. Behaviour and cortisol concentrations of castrated and sterilized males as well as of the females were investigated for three weeks. The most important results were the following: There were almost no differences in baseline cortisol concentrations and cortisol reactivity between castrated and vasectomised males. In addition, we did not find significant differences in basal cortisol concentrations between females living together with castrated or vasectomised males. Vasectomised and castrated males, however, differed in their total amount of sexual behaviour as well as in different sociopositive behavioural patterns. No significant differences in agonistic or play behaviour were found. The females living together with the vasectomised males exhibited significantly more agonistic behaviours towards the males than the females cohabitating with the castrated males. An analysis of the dominance hierarchies shows that in two groups, castrated males were potentially included into the female dominance hierarchy. Even though there were hardly any differences in terms of cortisol concentrations, the results regarding the behaviour could be interpreted as signs for potentially impaired welfare of the castrated males. An interesting finding is the potential inclusion of castrated male guinea pigs into the female dominance hierarchies, which would definitely represent a major change of the usual dominance hierarchy, and would probably be linked to worse welfare for the affected male.

Inbreeding effects in the West African cichlid fish *Pelvicachromis pulcher*

Alexander J. R. Keller; Rieke F. Schons; Simon Vitt; Timo Thünken

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Inbreeding is defined as mating between related individuals and is often avoided in the animal kingdom because it increases the genetic homozygosity and therefore can promote the expression of recessive deleterious alleles. The reduced fitness of inbred offspring is known as inbreeding depression. Despite the costs of inbreeding, some animal species tolerate or even prefer mating with kin. One example for such an inbreeding preference was found in an inbred population of the West African cichlid *Pelvicachromis taeniatus*. In this species, kin-mating preferences may be adaptive due to kin-selected benefits and the absence of strong inbreeding depression. The goal of the present study was to examine inbreeding effects in a genetically diverse lab population of the closely related species *Pelvicachromis pulcher*. We found already evidence of inbreeding depression in F1-inbred juvenile fish. Here we focussed on the impact of inbreeding on reproductive traits in adult fish and on resulting F2 offspring until two days after hatching. In a previous study we found already evidence of inbreeding depression in F1-inbred juvenile fish concerning survival and growth. Our results showed clear signs of inbreeding depression, but the effects were less pronounced in comparison to the F1 generation.

Nematoda Hox gene complements and cluster evolution

Joseph Kirangwa

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One of the most striking shared developmental mechanism in the divergent animal phyla is the patterning of the anterior-posterior axis by the highly conserved homeodomain-containing transcription factors encoded by hox genes. Traditionally nematodes are regarded as different because *C. elegans* is different. The Hox cluster in *C. elegans* has six Hox genes in three paralogous groups: one anterior Hox gene, ceh-13, two middle group paralogs, lin-39 and mab-5, and three posterior paralogs, egl-5, nob-1 and php-3. ceh-13, lin-39, mab-5 and egl-5 are in one cluster with ~300 kb interval and are interspersed by many non-Hox genes while the additional php-3 and nob-1 are located ~4 Mb away on the same chromosome III. Here, I re-visit the Hox gene complements of Nematoda not only to understand the evolution of this key set of body pattern genes using new available high-quality genomes spanning the Nematoda but also to classify the loci into their orthologue groups as well as looking for linkage between loci. I used a sequence similarity-based search pipeline BITACORA to find hox genes in 110 nematode genomes spanning Nematoda, mafft for sequence alignment, iqtree2 for phylogenetic reconstruction and SimpleSynteny to infer hox gene order and orientation in scaffolds. I found hox gene complement across the nematode tree very dynamic. lin-39, ceh-13, mab-5, egl-5 and php-3 were universally present across the Nematoda. Hox3 was present in Clade I-III but found missing in Clade IV-V of Nematoda. nob-1 posterior paralog present *C. elegans* was found missing in early branching caenorhabditis species clade including the rest of the Clades across the phylum. I found a losely organized hox cluster in different nematode genomes across the phylum. However, *Plectus sambesii* had a strikingly dense hox cluster. The hox genes ceh-13, lin-39, Hox3, mab-5 and egl-5 are in the same cluster which is ~35 kb. php-3 is located approximately ~400 kb away on a different contig but potentially on the same chromosome with the rest of the core hox genes. Clade I and II species showed disintegrated hox gene clusters. Hox gene evolution is highly dynamic. Highly contiguous nematode genomes enables Hox cluster size identification, gene order and orientation. The hox gene reference atlas generated will complement future functional studies and annotation of hox genes of novel non-model nematode genomes.

Identification and functional analysis of genes involved in the hypoxia tolerance of the whale brain

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The mammalian brain is dependent on continuous supply of oxygen. Reduced oxygen availability may have a devastating impact on brain function and neuronal survival. Due to their repetitive breath-hold diving and resurfacing behavior, diving mammals like whales and seals are routinely exposed to low oxygen availability and oxidative stress caused by reperfusion after the dive. Some whales can dive for two hours or more, evidently without any brain damage. Physiological adaptations of diving mammals have been investigated extensively. They include enhanced oxygen storage and transportation capacity and the ability to reduce energy consumption during diving via cardiovascular adjustments, i.e. bradycardia and peripheral vasoconstriction. When the vasoconstrictor response is fully activated, most organs and tissues experience a shortage of oxygen, while the much-reduced cardiac output of blood is distributed mainly to the brain. Despite these measures, arterial pO₂ of whales may fall below the critical oxygen tension threshold for ischemic brain damage. To survive such low O₂ levels without impairment of cerebral function, additional adaptations on the molecular level are required. However, the molecular mechanisms protecting the whale brain from hypoxic injuries and reoxygenation events are largely unknown. To reveal basic molecular differences in the brain of whales and their closest terrestrial relative, the cattle, we compared brain transcriptomes of the killer whale (*Orcinus orca*), the long-finned pilot whale (*Globicephala melas*), the minke whale (*Balaenoptera acutorostrata*) and the bowhead whale (*Balaena mysticetus*) with brain transcriptomes of the cattle (*Bos taurus*). The effect of selected genes with marked differential expression between whales and cattle were tested in neuronal mouse cells that were exposed to normoxia, hypoxia and oxidative stress. Our results indicate adaptations to survive low oxygen conditions in the whale brain on the molecular level.

„feeding vs. starvation“ – Neuropeptidomics of the central nervous system of *Drosophila melanogaster* L3-larvae

Anna-Sophie Kügler; Susanne Neupert

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Starvation is induced by a lack of nutrients and energy and drives eating and food-seeking behavior. In addition, hunger is a guidance signal that ensures that the search for food only takes place when needed. This occurs via neuronal signals such as neuropeptides. Neuropeptides are shorter peptide molecules of a length up to 45 aa produced by neuronal and endocrine cells. They are processed from larger preproproteins that contain a signal peptide and canonical prohormone convertase processing sites, and executes functions as neuromodulator or hormone via G-protein-coupled receptor (GPCR)-signaling. To uncover potential peptidergic candidates which are involved in the regulation of feeding, we used tissue extract analysis by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS and Q-Exactive Orbitrap mass spectrometry (MS) to investigate the neuropeptidome of L3 *Drosophila* larvae under fed and starved condition. Therefore, CNS samples from three different test groups are tested: (1) non-starved, (2) 24 hour starved, and (3) 48 hours starved larvae. Statistical comparison of resulting mass spectra revealed changes in the ion signal intensities of products of eight neuropeptide genes (sif amide, short neuropeptide F, extended fmrfamide, hugin, calcitonin-like diuretic hormone-31 [DH-31], allatostatine-C, corticotropin releasing factor-like diuretic hormone-44 [DH-44], kinin). By applying Q-Exactive Orbitrap MS, we could confirm the amino acid composition of these neuropeptides by fragmentation analysis. In addition, we found dynamics in the intensity of ion signal under different feeding conditions which are not match with known *Drosophila* neuropeptides. The sequence of the molecules has to be characterize in further experiments using de novo sequencing and BLAST data bank search. The results of our study provide necessary input for future measurements up to single cell level to study the dynamic in up- and downregulation of neuropeptides underlying mechanisms regulating feeding.

Airway specific inhibition of glutathione peroxidase as well as activation of Nrf2 increases lifespan in a *Drosophila* COPD model

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Chronic obstructive pulmonary disease (COPD) is a syndrome encompassing different chronic lung diseases and it is the third leading cause of death worldwide. To model COPD in *Drosophila*, animals were chronically exposed to cigarette smoke, which induced major hallmarks of the disease including structural changes of the airways and a reduced lifespan. Glutathione metabolism and the Nrf2 signaling pathway are known to mitigate the negative effects of cigarette smoke and thus counteract early COPD pathogenesis. The transcription factor Nrf2, which is negatively regulated by Keap1, controls expression of major enzymatic antioxidants including those of the glutathione metabolism. Here, Nrf2 was ectopically activated via tissue specific CRISPR/Cas9 of keap1 silencing exclusively in the airway system. Smoke exposed flies died significantly faster than control flies, but activation of Nrf2 exclusively in the trachea rescued most of this reduced lifespan phenotype. Moreover, CS exposure caused higher mortality rates after low oxygen treatment, while ectopic Nrf2 activation also rescued this phenotype partially. Interference with the Nrf2 signaling pathway also affects downstream targets including the glutathione metabolism. Glutathione peroxidases (gpx) catalyze the conjugation of reduced glutathione (gsh) to hydrogenperoxide (H_2O_2), leading to the reduction of H_2O_2 at the cost of reducing the pool of reduced glutathione. Silencing of gpx in the airways leads to higher cellular GSH/GSSG ratios and, more importantly, increases lifespan of CS treated flies. Taken together, activation of Nrf2 signaling and inhibition of glutathione peroxidase exclusively in the airway epithelium could be an effective treatment strategy for COPD patients that should be explored in future studies.

The secretory pathway protein Sec31 controls composition and function of the presynaptic active zone

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Information processing in the nervous system is shaped by neurotransmitter release from synaptic vesicles (SVs) at the presynaptic active zone. The cytomatrix at the active zone (CAZ) controls exocytosis by physically coupling SVs to release-sites. Correspondingly, the protein composition of the CAZ is functionally highly relevant and disrupting its molecular organization impairs synaptic transmission. However, the molecular mechanisms governing CAZ assembly are incompletely understood. The ELKS/CAST homolog Bruchpilot (Brp) is a core CAZ component in *Drosophila melanogaster*, which supports neurotransmitter release by tethering SVs and clustering voltage-gated calcium channels at the active zone. Using a short C-terminal Brp fragment as bait we found a strong enrichment of the secretory pathway protein Sec31 in affinity purification assays. Here, we further investigated this interaction by combining super-resolution microscopy and electrophysiology. Consistent with the biochemical results, Sec31 and Brp colocalize within the neuronal endoplasmatic reticulum. RNA interference mediated knock-down and mosaic knock-out of Sec31 in motoneurons reduces Brp expression at neuromuscular CAZs. This is accompanied by decreased active zone levels of voltage-gated calcium channels and the priming protein Unc13A. As a result, neurotransmitter release probability drops and synaptic efficacy is reduced. We conclude that Sec31 activity influences CAZ assembly and thereby impacts presynaptic function.

The myology of the crocodile lizard, *Shinisaurus crocodilurus* Ahl, 1930 – Part II: Description of the posterior extremity

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The crocodile lizard, *Shinisaurus crocodilurus* Ahl, 1930, is a rare and endangered squamate occurring in south-east China and north-east Vietnam. It is the only extant representative of the Shinisauridae, which are an early offshoot of the anguimorph clade that includes the earless as well as the true monitor lizards. The overall phylogenetic relationships within anguimorphs remain controversial when morphological and molecular data are compared, especially regarding the placement of the Helodermatidae, and whether they belong to the aforementioned clade or not. The majority of morphological data so far are based on the skeletal system only, whereas information on soft tissues is rather sparse. The present study forms the initial part of a broader approach to anguimorph morphology that will focus on the comparative myology of a number of species, including helodermatids and varanids. This particular subproject deals with the posterior extremity of *S. crocodilurus*. A total of 6 alcohol-preserved specimens (snout-vent length 135-156 mm) were manually dissected for the present study. The specimens were skinned and each individual muscle was isolated by scalpel and spatula until the bone was reached. Consecutive numbers were assigned to each of the resulting muscle bellies, totalling to 101 separate units whose origin and insertion was documented photographically and by drawings. These descriptive data now will serve the basis for a future comparative approach.

Adaptation of olfactory brain regions in Hemiptera in transition from land to water habitats

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Hemiptera comprise a large group of insects with diverse morphology and lifestyles inhabiting a diversity of terrestrial and aquatic ecosystems. As an adaptation to freshwater habitats, the backswimmer *Notonecta glauca* (Notonectidae), the creeping bug *Ilyocoris cimicoides*, and the benthic *Aphelocheirus aestivalis* (both Naucoroidea) exhibit a range of respiratory systems (air bubble -> plastron) and swimming apparatuses (1). In a comparative study, we employ neuron tracing and volumetric measurements of brain neuropils in true water bugs (Heteroptera, Nepomorpha) with respect to their olfactory pathway as opposed to land-living relatives, the firebug, *Pyrrhocoris apterus* and the milkweed bug, *Oncopeltus fasciatus*. Common brain features of aquatic and terrestrial bugs are a portioned antennal lobe (AL) including glomeruli structures and a mushroom body (MB) formed by small globuli cells. In contrast to land-living bugs, all studied species of water bugs are devoid of an MB calyx, as previously reported (2), but both groups form a complex multi-lobed peduncle. In *N. glauca* the MB is composed of ~ 5000 (n=2), whereas in a terrestrial species (*O. fasciatus*) we found ~ 2000 globuli cells. *N. glauca* has a dimorphic antennal lobe with about 35 glomeruli in females, and 30 glomeruli in males (n=1), some of them supplied by multiglomerular sensory neurons. In aquatic bugs, glomeruli are not as distinct as in terrestrial species, and olfactory neuropils are reduced in volume. The AL occupies ~ 2% and the MB up to 4% of the volume the central brain. Contrarily the relative size of putative olfactory structures of the terrestrial bug *O. fasciatus* comprise 20% for the AL and 10% for the MB. In large-eyed aquatic species, *N. glauca* and *Corixa punctata* (Corixidae) the optic lobes (OL) contribute up to 70% of protocerebral neuropil. Interestingly the Heteroptera species in our study, aquatic and terrestrial, have a all central body about 3% in size relative to the central brain. The question of how neural information is relayed from sensory neuropils, (AL and OL) in the protocerebral lobe in aquatic species, without an MB calyx remains open. Reduced olfactory neuropils in aquatic species might be due to a reduced perceptual odor space under water. Alternatively, omitting calycal pathways allows for a straight olfactory processing. Currently, we are thus focusing on identifying fiber tracts that connect sensory neuropils with the central brain.

Saltatory axonal conduction in the avian retina

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In contrast to most parts of the vertebrate nervous system, the ganglion cell axons in the retina typically lack any myelination. Ganglion cell axons of most species only become myelinated once they leave the retina to form the optic nerve. The avian retina is a well known exception in that ganglion cell axons are partly myelinated in the retinal nerve fiber layer. However, the functional and structural properties of myelination in the nerve fiber layer remain elusive. Here, we used large-scale multi-electrode array recordings in combination with immunohistochemistry and fluorescence microscopy to study the retinal myelination. Intraretinal myelination was accompanied by the formation of nodes of Ranvier. The internode length was positively correlated with the axon diameter. The variability of internode lengths along each axon was significantly smaller than across axons. Saltatory conduction of action potentials was observed in a large population of recorded cells. On average, myelinated axons had higher conduction velocities than unmyelinated axons while both groups showed a significant overlap at low velocities. The number of simultaneously active nodes was positively correlated with the conduction velocity. In contrast, the internode length and the time it took a node to activate were weak predictors for the conduction velocity. However in summary, the conduction velocity was well described by the number of activated nodes, the internode length, and the activation time in concert. The saltatory conduction in the avian retina resulted in higher velocities in comparison to the mammalian retina. In addition, we observed a large interspecific diversity of conduction velocities in birds that aligned with their respective ecology.

Extracellular carbonic anhydrase activity promotes a carbon concentration mechanism in metazoan calcifying cells

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Many calcifying organisms utilize metabolic CO₂ to generate CaCO₃ minerals to harden their shells and skeletons. Carbonic anhydrases are evolutionary ancient enzymes that were proposed to play a key role in the calcification process with the underlying mechanisms being little understood. Here we used the calcifying primary mesenchyme cells of the sea urchin larva to study the role of cytosolic (iCAs) and extracellular carbonic anhydrases (eCAs) in the cellular carbon concentration mechanism (CCM). Molecular analyses identified iCAs and eCAs in PMCs and highlight the prominent expression of a GPI-anchored membrane-bound CA (Cara7). Intracellular pH recordings in combination with CO₂ pulse experiments demonstrated iCA activity in PMCs. iCA activity measurements together with pharmacological approaches revealed an opposing contribution of iCAs and eCAs on the CCM. H⁺-selective electrodes were used to demonstrate eCA catalyzed CO₂ hydration rates at the cell surface. Knock-down of Cara7 reduced extracellular CO₂ hydration rates accompanied by impaired formation of specific skeletal segments. Finally, reduced pHi regulatory capacities during inhibition and knock-down of Cara7 underline a role of this eCA in cellular HCO₃⁻ uptake. This work revealed the function of carbonic anhydrases in the cellular CCM of a marine calcifying animal. Extracellular hydration of metabolic CO₂ by Cara7 coupled to HCO₃⁻ uptake mechanisms mitigates the loss of carbon and reduces the cellular proton load during the mineralization process. The findings of this work provide insights into the cellular mechanisms of an ancient biological process that is capable of utilizing CO₂ to generate a versatile construction material.

Forward masking of brainstem lateral line units of goldfish, *Carassius auratus*

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Forward masking is a form of sensory adaptation that is characterized neuronally by a reduced response to a probe stimulus that is preceded by a masker stimulus. While this has been particularly well studied in auditory neurons of vertebrates, there is up to date no account of forward masking for the fish lateral line system. We investigated forward masking in single lateral line neurons of the brainstem Medial Octavolateral Nucleus (MON) of goldfish using a double stimulus paradigm. Stimuli were sinusoidal water motions (50 Hz and 100 Hz, 500 ms duration, 100 ms rise/fall times) generated by a vibrating sphere (8 mm diameter). We first determined the temporal separation between two identical stimuli that is necessary to elicit forward masking. Stimuli had a fixed level (vibration amplitude: 159 μ m at 100 Hz/ 336 μ m at 50 Hz) with time intervals between masker and probe stimuli of 0, 20, 50, 100, 200, 500 and 1000 ms. A response ratio (RR) was calculated as the ratio of the responses to the probe relative to the responses to the masker stimulus. In 40 out of 63 (63 %) of the recorded units, responses to the probe were distinctly reduced compared to the responses to the masker for time intervals up to 200 ms, indicating forward masking. In these units, average RRs ranged between 0.5 at 0 ms and 0.67 at 200 ms. At 500 ms and 1000 ms time interval responses to probe and masker stimuli were near identical (average RRs 0.88 and 0.94). In 23 units (37 %), responses to the probe were not appreciably reduced at any of time intervals used (average RRs between 0.84 and 1.15), i.e., these units did not exhibit forward masking. We secondly examined if forward masking can be abolished by increasing the amplitude of the probe stimulus. Here, the time interval between the two stimuli was kept constant at 100 ms. The effect of forward masking was eliminated by increasing the amplitude of the probe stimulus by 5 dB. Further increases of the probe level resulted in stronger responses to the probe than to the masker (RRs > 1). Our data show that brainstem lateral line units can exhibit forward masking. We assume that this is due to adaptation of MON units to the masker stimulus and that the units are still in an adapted condition when the probe stimulus is presented shortly thereafter.

Impact of microbiome on animal development. Role of bacteria in early embryogenesis in *Hydra*

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Over the last few years, the role of the microbiome in host development, fitness and behaviour, was studied extensively. A healthy microbiome is essential for the normal development and maturation of the immune system, the nervous system, and host metabolic functions. Animals lacking their microbial partners show altered neuronal development and behaviour compared to control individuals harbouring a normal microbiome. In addition, the maternal microbiota appears to play a significant role in the offspring's protection against potential pathogens. It also contributes to that offspring selecting specific bacterial species that would later shape their own microbiome. In *Hydra*, a freshwater polyp of the Cnidarians, maternal antimicrobial peptides (AMPs) maintain the homeostasis between the bacterial colonizers and the epithelium of the early development stages, which fulfil a protective function of associated microbes for the developing embryo. Here, we aim to observe early embryogenesis in the freshwater polyp *Hydra* under germ-free conditions. Germ-free cultures were generated by treating polyps with antibiotics and then kept in sterile *Hydra*- medium. Embryos produced in these cultures were collected and observed daily until hatching. Early observations of the germ-free hatchlings show prolonged developmental period (delayed hatching) and lower hatching rate compared to control embryos produced under conventional conditions. Confocal microscopy images of germ-free hatchlings of neuronal marker proteins show fewer number of nerve cells compared to control hatchlings. However, this deviation in both rate and time of hatching was not observed when already developed embryos from conventional cultures were rendered germ-free and only hatched in germ-free conditions.

Visual Orientation towards static objects in the stick insect *Carausius morosus*

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Insects show robust visual orientation behavior towards static objects even without net image shifts on the retina (Varù, 1975, *Nature*). Kalmus (1937, *Z. vergl. Physiol.*) described what he called photohorotaxis, i.e., orientation towards black-and-white edges in *C. morosus* larvae. We have replicated this result for adult *C. morosus* and Zeng et al. (2020, *J. Exp. Biol.*) found similar behavior in larvae of *Extatosoma tiaratum*. The latter study also reports phototaxis in the absence of contrast edges. When walking towards black bars with less than 90° width, gypsy moths show a directional preference towards the dark center, whereas this preference shifts towards the high contrast edges for broader bars (Preiss & Kramer, 1984, *J. Comp. Physiol.*). Here, we test the effect of static image luminance and contrast on visual orientation in walking stick insects.

Given that both phototactic and edge orientation mechanisms may contribute to visual orientation towards dark bars, we attempt to disentangle their respective contribution by testing a set of six static visual patterns that differed in the number and contrast of edges. In addition to a 90° black bar (2 edges) and Gaussian luminance patterns (no edge) of 90° or 180° width, we used a “single-edge” stimulus consisting of a sharp edge next to a black-to-white luminance gradient (90°). Also, we used patterns with two edges separated by two linear gradients, such that the edges differed in mean luminance but not in contrast (135° width). Patterns were projected on a circular screen (20 cm height) surrounding an arena with a diameter of 120 cm. Behavioral data of freely walking, adult female *C. morosus* was collected as top-view videos. Head, thorax and leg trajectories were extracted through marker-less tracking. For Gaussian patterns, animals preferred the dark center over the regions of steepest change in luminance. In the presence of an edge, animals preferably walked towards these high-contrast regions. The type of stimulus had no impact on walking speed, but total walking distance increased for trials in which no edge was present. So far, we cannot predict which one of two edges will be chosen in a particular trial. This may indicate a subordinate role of luminance in cue conflict situations. At the same time, luminance alone predicts visual orientation behavior for patterns without edges.

CFTR function is restored by transfection with wtCFTR-mRNA via chitosan-based nanocapsules

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Cystic fibrosis (CF) is caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene that codes for a chloride channel. It is the most common genetic disorder in the Caucasian population. Decreased CFTR function leads to an imbalance in homeostasis of ion and water transport in secretory epithelia. Particularly in the lung, this defect impairs mucociliary clearance leading to the common clinical picture. Here we tested a state-of-the-art Ussing chamber named Multi Transepithelial Current Clamp (MTECC) for its suitability to record changes in conductivity via CFTR. Primary cell samples were collected by nasal brushings. The epithelial cells were cultured at an air-liquid interface and CFTR activity was evaluated after cAMP application. This culturing system proved to be resilient enough for multiple measurements of the same material. Transmembrane resistance remained constant over three months. In a first attempt CF cell were transfected with wtCFTR-mRNA via the commercially available Lipofectamine™. No significant improvement of the CFTR activation was recorded in the MTECC Ussing chamber. However, when chitosan-based nanocapsules were used to introduce the nucleic acid to the cells cAMP-induced activity of CFTR measured as transepithelial conductance was drastically improved (from $0,03 \pm 0,26$ to $0,93 \pm 0,45$ mS/cm²). The effect could be observed over a period of more than 48 h after transfection ($p \leq 0,0001$). These results distinguish the chitosan-based nanocapsules as a new and more efficient way for mRNA transfections. A future use in drug delivery seems very feasible.

A monotypic genus? Revision of *Exocnophila* stick insects (Phasmatodea) unveils 17 species

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Recent studies have demonstrated that the mesodiverse lineage of stick and leaf insects (Phasmatodea) comprises a high amount of hidden diversity with new taxa frequently being discovered. South America appears to be one of the hotspots for phasmatodean diversity but remains largely uninvestigated. *Exocnophila* is a recently erected genus of apterous Brazilian stick insects (Phasmatodea) currently only known from females pertaining to a single species. A primary analysis of specimens collected in expeditions to several regions of Brazil, however, pointed to a much higher diversity potentially including several new species. In order to review the genus, we performed extensive morphological examinations and compared our findings to the original description of the type material. We present a new diagnosis for the genus, including the description of the male as well as detailed characteristics for both sexes and the egg. Consequently, we were able to recognise at least seventeen species of *Exocnophila*, all occurring throughout the Atlantic Forest and Caatinga in Brazil. Furthermore, we obtained molecular data for several species to infer their phylogenetic relationships and to confirm their status. Morphologically, species of *Exocnophila* are generally similar to each other, mostly being diagnosed by a discrete set of characteristics of both sexes and eggs. Surprisingly, the morphology of male genitalia is similar among some species and cannot be solely used for diagnosis. By contrast, the molecular data was found to clearly be able to delimit the proposed species. Our biological observations for several species showed that *Exocnophila* are mostly ground-dwellers, slow-paced and feed on a variety of plants. When disturbed, specimens will stop moving and frequently release a minty scent, while a few can also spew. Two species are found to reproduce asexually with no known male. In conclusion, the example of *Exocnophila* emphasises the need for further sampling efforts and morphological analyses coupled with phylogenetic delimitation inferences to reveal more of the underestimated diversity in Brazil and South America.

Head without a brain: The effect of head miniaturization and modification on the nervous system of fluid feeding millipedes

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The colobognathan millipedes have highly modified heads, with a complex sucking pump, which enables them to feed on liquids. In some Colobognatha - Polyzoniida and Siphonocryptida - the conical heads are extremely small, and the internal volume of the head is mainly occupied by musculature and the mouthparts. It was unknown how such modifications influenced other cephalic organs like the nervous system. Here we used x-ray tomographic microscopy and histology to study the morphology of the nervous system of 4 polyzoniidan and 2 siphonocryptidan species, spanning all currently recognized families of the group. We show that in all studied Polyzoniida and Siphonocryptida the brain is located in the anterior body-rings instead of the head. Indeed, the brain is larger than the head in several representatives. The brain is highly condensed compared to other millipedes, with neither a clear division into proto-, trito- and deutocerebrum, nor suboesophageal ganglion. Furthermore, the ventral nerve chord has a uniform diameter without clear ganglionic swellings. We suggest that the shift of the brain from the head into the anterior body-rings is the result of the strong miniaturization and modification of the head. A similar situation can be found in other minute arthropods, like pauropods, insects, and spiders, in which the brain, or parts of it, shifted into the body or even into appendages. Our results show that not only miniaturization of the whole body but also changes in the shape and size of the head, related to specialized feeding habits, can strongly influence the morphology of the nervous system. Based on the structure of the nervous system and a number of other morphological characters we suggest that Polyzoniida and Siphonocryptida form a monophyletic taxon.

Walking bumblebees see faster

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Neuronal information processing is metabolically costly and it is evolutionary advantageous to limit these costs to the necessary minimum through state dependent modulation of activity. For example, motion sensitive neurons in flies have a decreased gain, when the animals sit as opposed to flying (Maimon et al. 2010) or walking (Chiappe et al. 2010). So far, it is not known if locomotor activity also changes the response properties of the photoreceptors. To address this question, we compared electroretinograms (ERGs) from the compound eyes of tethered bumblebees that were sitting or walking on an air supported ball. We stimulated with a Gaussian white noise signal delivered by a green (530 nm) LED. To quantify the response delay with respect to the stimulus, we calculated the cross-correlation between the stimulus signal and the ERG. The delay dropped from 9.0 ± 0.75 ms in sitting animals to 7.4 ± 1.0 ms in walking animals. To assess which stimulus frequencies are affected by the changes we calculated the linear coherence of the system and found a shift to higher frequencies in walking animals. Using a thermographic camera, we were able to show that the increase in response speed can be observed in sync with an increase in head temperature. We tested if temperature changes are sufficient to explain our observation by heating up sitting bumblebees with a heat lamp. We found similar changes in ERG delay as during walking. We conclude that heat generated by the animals during walking helps them to process visual stimuli faster, which might be necessary for a moving animal.

When it gets warm in winter: Phenotypic plasticity in a cold adapted population of a range expanding spider

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The spatial distribution of many species is currently shifting since species track favorable environmental conditions due to climate change. The European wasp spider *Argiope bruennichi* has undergone a rapid latitudinal range expansion from the Mediterranean region into the Baltic states and Scandinavia – however, faster than the climate changed. Previous studies showed that hatched spiderlings from edge populations prefer colder temperatures when given a choice and show higher survival probability under the coldest exposure temperatures, strongly suggesting local adaptation to colder winter conditions. However, edge spiderlings weigh less than core spiderlings, and all lose weight during a warm winter treatment. Populations at the northern edge of the distribution might therefore suffer disproportionately from increasingly warmer winters. To explore the degree and limits of plasticity under warmer winters in a cold-adapted population, we exposed spiderlings from Estonia to cold, moderate, and warm winter conditions. We collected data on temperature effects on 1) survival, 2) lipid content, 3) metabolomics, and 4) gene expression. We can build up on a high-quality reference genome and focus on differential expression of cell repair genes, heat shock proteins, and higher expression of cryoprotectants. Studying the responses to different winter temperature regimes will provide information on the degree and mechanisms of plasticity and help predict responses to climate change of recently cold-adapted populations.

Potential neurophysiological mechanism underlying pCO₂ dependent impairment of inducible defenses in *Daphnia pulex*

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Concentrations of dissolved CO₂ are continuously increasing in aquatic habitats worldwide and this is typically accompanied by acidification. These processes and their effect on marine organisms have been well studied reducing calcification rates, growth and fitness. Some marine species are even severely impaired in their ability to sense important environmental cues, thereby reducing their potential of e.g. behavioral adaptations to predators. This is discussed to be due to changes in acid-base regulation through which intracellular ion concentrations are changed rendering inhibitor GABAergic signals excitatory. Similarly, freshwater species have been described to be affected by elevated levels of pCO₂. The freshwater crustacean *Daphnia pulex* e.g. is reduced in its ability to develop morphological defenses, thereby becoming more vulnerable to predators. Also here, changes in the underlying physiology have been discussed. However, the neurophysiological background behind these changes remains largely unknown. We performed a full-factorial experiment, using a control condition in comparison to CO₂ exposed, kairomone exposed and CO₂ + kairomone exposed *D. pulex*. We then analyzed CO₂ depended changes in gene expression patterns focusing on those genes that are involved in acid-base regulation and GABAergic signaling. From these, two ion-cotransporter candidates were functionally tested with the help of selective ion-cotransporter inhibitors. We find that the potassium-2-chloride co-transporter is significantly down-regulated under elevated levels of pCO₂. The selective inhibition of this co-transporter with the loop diuretic furosemide showed a significant reduction of neckteeth expression. We conclude that furosemide mimics the CO₂ dependent neuronal deficits. This leads to an increased intracellular chloride concentration by which gabaergic actions could indeed become excitatory. We here provide a first insight into the possible neurophysiological background underlying the CO₂ dependent impairment of inducible defenses in a freshwater species.

Population history reconstruction of eukaryotes from genomes

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Events that shaped a population in the past, such as migration or bottlenecks, are important to understand, as they add evolutionary and ecological context to a species' dispersal and its genetic diversity. The evolutionary history of a species is recorded in the pool of genomic sequences from individuals of a population. Reading this information might help to predict future evolutionary trajectories of a species and can aid to structure conservation strategies. The identification of patterns of genetic variation in the genome allow inferences about past population demography. Models based on the sequentially Markovian coalescent (SMC) framework were developed to match these variations to past population history. However, these models can be biased by the genome assemblies used for analysis or by complex traits of a non-model organism, like reproductive mode, dormancy, mutation and recombination rates or ploidy. Here we investigated 1) how the quality of data and prior knowledge about the biology of the organism and 2) how the SMC model used affected the reconstruction of population history, and 3) whether the inferred population history matched to past climate data. For our tests we used resequencing data of the pan-European non-biting midge *Chironomus riparius*, and two nematode species, the triploid parthenogenetic *Panagrolaimus kolymensis* and a diploid hermaphrodite, *Propanagrolaimus* sp. JU765. I found that the reliability of population history derived using the multiple sequentially Markovian coalescent (MSMC2) increased with a high-quality genome of *C. riparius* compared to previous studies. Estimates were consistent with past climatic events, possibly explaining a spread of this midge in Europe during the transition of Pleistocene to Holocene. Estimates of the ecological sequentially Markovian coalescent (eSMC2) for both nematode strains proved less robust due to uncertainties in recombination or mutation rates and lower quality of draft genomes compared to *C. riparius*. SMC model violations related to polyploidy and low recombination rates proved to be particularly difficult. Inferences with eSMC2 resulted in partially comparable estimates to MSMC2. Overall, these results show the power of SMC models when sufficient data and additional biological information is available. My results indicate that SMC models need to be adapted to work better with challenging non-model organisms.

Characterization and functional analysis of ABCB transporters in the leaf beetle *Chrysochus asclepiadeus*

Cäcilia Plate; Paulina Kowalski; Susanne Dobler

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Some plants produce cardenolides to protect themselves against predators. These toxins act by inhibiting the Na,K- ATPase. Nevertheless, various insects have adapted to cardenolides in their food and even sequester them. In this process, cardenolides are recycled and used as anti-predator defense. In the leaf beetle genus *Chrysochus*, some species are adapted to cardenolides *C. auratus* and *C. copaltinus* while others feed on plants devoid of cardenolides *C. asclepiadeus*. In a previous study, a radiolabeled cardenolide was transported into the defense fluid in the adapted species, but eliminated with the feces in *C. asclepiadeus*. We hypothesized that efflux transporters of the ABCB protein family coordinate this transport and could confirm this assumption in *C. auratus*. In this study we analyze the situation in *C. asclepiadeus* to determine how many ABCB gene copies exist in *C. asclepiadeus*, how similar their tissue and substrate specificity is to the situation in *C. auratus*, and at which point in the evolution of the group adaptation to cardenolides occurred. In the non-adapted species, we could bioinformatically identify two ABCB full transporters (ABCB1 and ABCB2), whereas the adapted species has three ABCB full transporters (ABCB1-3). The ABCB proteins were successfully expressed in insect cells and confirmed immunologically with the specific antibody C219. ATPase activity assays suggest that ABCB1 interacts with digoxin but not with ouabain and cymarin. In contrast ABCB2 could be stimulated by all three cardenolides tested. The tissue-specific expression by qRT-PCR showed that ABCB1 is more strongly expressed in all tissues except for the Malpighian tubules. The major expression site of ABCB1 is in the nervous tissue, thus suggesting a protective role in the blood-brain barrier that we previously postulated. In contrast the highest expression of ABCB2 was in the Malpighian tubules, suggesting a role in rapid excretion of toxins. Compared with *C. auratus*, the tissue-specific expression of the two genes of *C. asclepiadeus* is much lower. The main expression of ABCB2 of *C. auratus* was in the elytra, where the defense glands are located, and its specific interaction with the host plant cardenolide cymarin supports the involvement of this ABCB transporter in adaptation to cardenolides. This adaptation apparently arose in conjunction with a gene duplication after the split from the ancestor of *C. asclepiadeus*.

Rodeo on aquatic mammals: surface adaptations of arthropod parasites to underwater attachment

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Marine mammals host a great variety of endo- and ectoparasites, which are adapted to their hosts in a co-evolutionary arms-race. However, only little is known about the biology of marine mammal arthropod parasites, but even less about physical aspects of their life in such a challenging environment. We hypothesized that the exoskeleton material and the cuticular structures of the seal lice (*Echinophthirius horridus*; Anoplura; Insecta), whale lice (*Isocyamus deltobranchium*; Cyamidae; Amphipoda; Crustacea), and naso-pharyngeal mite (*Halarachne halichoeri*; Arachnida; Acari) have evolved by adapting their morphology, surface structures and material properties to survive on gregarious and diving wildlife. By using micro-computed tomography (μ -CT) and scanning electron microscopy (SEM), we characterized anatomical specializations in these parasites to attachment, which enable their fixation to their hosts during dives, haul-out, currents, turbulence, social interactions. Furthermore, we determined rather remarkable adaptations in material composition of the cuticle, spiracles and ventral spines by histological methods and confocal laser scanning microscopy (CLSM). Additionally, we designed an experiment for mechanical characterization of attachment abilities of the parasites on the range of artificial and natural surfaces. Here we discuss convergent solutions to the challenges connected with marine wildlife as hosts and numerous structure-function relationships in the parasite attachment structures. Some results appear promising for transferring functional solutions from biological systems to materials science and engineering.

Plant-mediated indirect effects of drought and nitrogen fertilization on an insect herbivore

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Global environmental change poses a growing threat to biodiversity. Specifically, anthropogenic climate and land use change are key drivers of biodiversity loss. While their effects on biodiversity are well-documented, potential interactions among them are largely unknown. Here, we investigate the effects of drought and nitrogen fertilization, key components of climate and land use change, on host-plant quality and consequently on the preference and performance of the butterfly *Lycaena tityrus*. We found that insect performance was reduced by either a lack of or strong fertilization, potentially due to nitrogen limitation or increased level of toxic allelochemicals respectively. Nitrogen fertilization, though, had a positive effect on female oviposition preference, resulting in a mismatch between preference and offspring performance at high nitrogen levels. Drought and nitrogen deposition did show interactive effects on the plant chemical composition, but did not interact strongly to affect herbivore performance. Plant drought stress additionally reduced herbivore performance. Our results demonstrate changes in host-plant quality caused by variation in water and nitrogen availability, in turn indirectly affecting the herbivore. Our study highlights the importance of such indirect effects of global environmental change as a potential source of variation. Such effects should be considered in order to reduce uncertainties on the effects of global change on biodiversity.

Experimental study on the influence of fish presence on the macrozoobenthos of stream ecosystems.

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Aquatic invertebrates show a wide variety of reactions to changes in environmental conditions. One of these reactions is the drift, in which the animals rise into the water column and drift downstream. This drift behavior is used in the so-called ExStream System to investigate the behavior of invertebrates under various anthropogenic stressors. The ExStream system is a field-mesocosm setup that has already been used including in 2021 and 2022 on the Boye, a tributary of the Emscher, to investigated the influence of anthropogenic stressors on river ecosystems. Part of this study also investigates the influence of stressors on indirect and direct predation effects on macrozoobenthos. Many invertebrates use drift as an escape mechanism to evade predators like fish. As aquatic invertebrates are able to perceive chemical compounds from predators, termed as kairomones, kairomone-enriched water or fish predators were introduced into the mesocosms of the ExStream System. Designing a complementary experimental laboratory setup, we would like to find out at which kairomon concentration the invertebrates start drifting and whether a similar behaviour can also be observed in the direct presence of predators. This would greatly facilitate the investigation of predator-prey interactions in the future. In order to monitor the reactions of the invertebrates, the experimental setup is built from transparent Plexiglas tubes. In addition, the behavior of the macrozoobenthos is filmed to be evaluated in a computer-based assessment afterwards.

Aerial and underwater single target acuity in harbor seals *Phoca vitulina*

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Under numerous circumstances, the detection of single objects is vital for harbor seals when orienting on landmarks or stars at the water surface or when hunting prey or maybe even when using information from particle-induced optic flow under water. Thus we set out to investigate how small single objects must be to still be detectable by harbor seals. Aerial and underwater single target acuity (STA), defined as the target size the seal could detect with 75% correct choices at a viewing distance of 60cm, was assessed for stationary and moving single targets in two male harbor seals. Experiments took place in an experimental chamber which provided a constant ambient luminance of $80\text{cd}/\text{m}^2$. In a two-alternative-forced-choice discrimination task, the seals had to indicate whether the single target was either presented on the left or right side of the monitor. The size of the single targets was systematically changed to obtain the STA. Aerial STA for high contrast (Weber contrast = 0.97) stationary targets was determined as $0.31 \pm 0.03 \text{ deg}$ ($N=2$). When the contrast of target to background was lowered, STA slightly decreased finally reaching $0.35 \pm 0.03 \text{ deg}$ ($N=2$) for a target with a Weber contrast of 0.22. Aerial STA for high contrast moving targets amounted to $0.27 \pm 0.05 \text{ deg}$ ($N=2$) and again slightly decreased the lower the contrast. However, neither contrast nor motion had a significant impact on aerial STA ($p>0.05$). The STA in air was, however, worse than the grating acuity of harbor seals which can probably be explained by their rather low contrast sensitivity. Preliminary results from underwater testing for high contrast stationary targets suggest that underwater STA amounts to 0.32 deg and 0.74 deg in the two experimental animals. So, till now, at least one of the two animals already reached a similar underwater to aerial STA. Our STA estimates allow to assess the detection distance of single objects, for example for fishes underwater. Furthermore, the STA will be informative regarding simulations and behavioral experiments on optic flow that are inspired by the idea that aquatic animals induce optic flow when swimming through particle-rich water.

Old and new: micro-CT measurements of contrast enhanced nerve cells

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In this project, we combine the staining of nerve cells by heavy metal solutions with modern micro-CT measurements. In such a so-called "backfill", cut or scribed nerve endings are placed in a heavy metal solution for several hours, which then diffuses through the nerves. Nickel and cobalt chloride are to be used for such a backfill experiment, on the hypothesis that these substances are particularly radiopaque and can therefore be particularly highlighted in the X-ray image. The ears of grasshoppers will serve as the first experimental animals to test this method. Here, both directions, into the periphery and into the prothoracic ganglion, are to be stained and subsequently reconstructed. After reconstruction, various parameters, such as density and degree of branching, can be calculated and, in addition, a rendered 3D model can be rendered without cutting artifacts, which typically would occur with histological methods.

Temperature-dependent variability of detectable neuropeptide and aminergic titers in individual *Drosophila* neurons by quantitative immunolabelling

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Neuroactive substances like biogenic amines and neuropeptides play an essential role in the regulation of physiological processes and animal behaviors. Both are released from neurons and have highly diverse functions, structures and dynamic expression pattern in the nervous system. Physiological and behavioral experiments to understand their functional role within neuronal circuits often rely on animal immobilization by cooling; however, the influence of this intervention on the physiology of the organism remains largely unexplored for neuropeptides. In this study, we investigate cooling effects on individual corazonin (Crz)-expressing and octopaminergic (OA) neurons of larval and adult fruit fly *Drosophila melanogaster* by quantitative immunocytochemistry. Corazonin and octopamine are involved in a variety of neuronal circuits regulating e.g. feeding, growth and stress responses. To uncover temperature-depending effects, we used two test groups: (1) uncooled animals, and (2) animals cooled for 60 min. As a marker we used a gfp-guided Tcd2-Gal4 fly strain and performed whole-mount immunofluorescence stainings against octopamine (OA) and corazonin (Crz) on L3 larval CNSs and adult brains. Statistical comparison revealed higher signal intensities of Crz-ir and OA-ir larval and adult neurons in cooled flies compared to uncooled. In addition, our preliminary data indicated also a difference in the soma size of the dorsolateral Crz-processing neurons which showed a larger volume in cooled animals compared to uncooled. Our experiments suggest a non-negligible influence of the cooling time on the neuropeptide and aminergic titers. Thus, this aspect should be strongly considered for experimental designs of future studies to investigate the functional role of these signaling molecules within neuronal circuits.

Neuroglobin might be a protective factor under acute stress conditions in zebrafish

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Globins are small metalloproteins with the ability to bind gaseous ligands such as O₂ and NO. In vertebrates, eight globin types have been identified so far. Within these, hemoglobin and myoglobin are well-investigated proteins with important physiological functions in respiration and oxidative energy metabolism. The functional diversity of globins is great, and for some of the vertebrate globins the biological role is still inconclusive. This is also the case for neuroglobin (ngb), a phylogenetically ancient hexa-coordinated vertebrate globin. Ngb is a monomer and predominantly expressed in neuronal cells. It might function in O₂ supply, or prevent cells from reactive oxygen species (ROS) or nitrosative stress (RNS) induced damage. Studies also point to a neuroprotective role under ischemic conditions. Due to their aquatic lifestyle, fish are often exposed to low O₂ concentrations and in some species, it was shown that ngb expression correlates with hypoxia-tolerance. Our working group generated a zebrafish ngb^{-/-} mutant line using the CRISPR/Cas9 system. We broadly investigated the impact of the ngb^{-/-} knockout on zebrafish physiology *in vivo*. This included studies on the development, a phenotypic screen and transcriptome analysis of larvae at 72 hours post fertilization (hpf). Further, we employed hypoxia and cold stress experiments with ngb^{-/-} mutant larvae or ngb^{-/-} mutant adults to determine the stress tolerance of these zebrafish. In both conditions, we found increased vulnerability of the ngb-deficient zebrafish. Hypoxic stress at the time of neurogenesis (8-32 hpf) led to a metabolic shift towards glycolysis and promoted growth arrest. To elucidate the protective role(s) of Ngb during stress in adults, we are currently analyzing RNA-Seq data of brains from acute stressed and stress adapted ngb^{-/-} mutants. Initial results indicate adaptions regarding energy metabolism and elevated stress response. Our findings imply that Ngb may be involved in O₂ supply of neuronal cells and support a protective function for Ngb in zebrafish.

Functional morphology of filter-feeding in the Atlantic Mackerel (*Scomber scombrus*)

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Suspension feeders use a diversity of mechanisms to retain small particles from water for nutrition. Ram-feeding fishes create a feeding current through their forward motion and use cross-flow filtration to trap particles in their gill arch system. Thereby, the retained particles do not clog, keeping the separation medium functional. The morphology of the gill arch system in the ram-feeding fishes differs regarding the shape of gill rakers, mesh formation, and filtration area. The Atlantic Mackerel *Scomber scombrus* (Scombridae) stands out in this respect: Only the gill rakers at the first gill arch are elongated and typical for filter-feeders, the denticles are very long, reaching into the buccal cavity, and the teeth also form a mesh-like structure. We studied the flow patterns around this gill arch system and the influence of geometry, gill rakers, denticles, and teeth. Individuals were fixed in an open mouth position, scanned with μ CT, and 3D printed to be examined in a flow tank because it is complicated to observe the flow patterns in the buccal cavity of alive filter-feeding, normally schooling specimen. The streamlines, visualized with ink, encounter the anterior parts of the gill arch system at angles smaller than 45° indicating cross-flow filtration. However, the larger angles show dead-end filtration in the posterior region, where the particles accumulate between the denticles and the mucus before they are ingested. We suggest that this combination of mechanisms enables *S. scrombus* to retain particles smaller than the calculated mesh size. Additionally, the findings could be the basis for a biomimetic abstraction to develop filters for technical applications.

Functional Morphology and Ontogeny of the Swim Bladder–Vertebrae Association in *Pantodon buchholzi* Peters, 1876

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The freshwater butterfly fish *Pantodon buchholzi* Peters, 1876 is an obligatory air breather inhabiting oxygen-poor backwaters of tropical West Africa. Its dorsally flattened but ventrally keeled body, wing-like pelvic fins, and a highly specialised visual and lateral line system equip this surface dweller well for a life at the water-air boundary. Even more peculiar than its external morphology is the close association of the swim bladder with the vertebrae. In the precaudal vertebrae, the swim bladder penetrates the parapophyses, resulting in air spaces permeating a lattice-like framework of bone trabeculae. A presumed function in buoyancy control and density reduction of the axial skeleton, analogous to the postcranial skeletal pneumaticity (PSP) found in dinosaurs including birds, has been discussed early on, but a recent publication questioned whether these minute protrusions serve any function at all. We therefore compared the centres of mass (CoM) in digital models based on µCT scans of *P. buchholzi* in which the dorsal protrusions had been artificially removed with models where they were retained. A dorsal shift of the CoM is noticeable when protrusions were retained, which can be interpreted as a surface adaptation, likely contributing to the stabilisation of the fish below the water surface. In addition to the controversial functional aspects, the ontogeny of these air-filled vertebrae remains obscure. Histological sections of different growth stages showed that, regardless of the superficial resemblance of the adult condition to dinosaurian PSP, the underlying developmental processes differ. The invasion of the swim bladder protrusions is limited to long-persisting cartilaginous vertebral elements, which undergo endochondral ossification relatively late during ontogeny. Recesses resulting from cartilage resorption eventually become directly invaded by the protruding swim bladder.

Cats gaining ground: Evidence for European wildcat (*Felis sylvestris*) occurrence in an area with high importance for population linkage in central Germany

Michael Schulte; Laura Schulte

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After being on the brink of extinction in the 20th century, the populations of European wildcat (*Felis sylvestris*) are partly increasing again in Europe. In Germany the species survived in the regions Eifel and Harz Mountains in two relict populations, which today mark the main areas of its occurrence. Today, wildcats are spreading again from these two regions. Although the population is increasing, the species still remains on the red list as endangered and is listed in annex IV of the European habitat directive. Habitat fragmentation, traffic network, intensive agriculture and forestry as well as hybridization with domestic cats (*Felis catus*) and disease transmission are considered the main threats for wildcats. In the Teutoburg forest between Bielefeld and Detmold, the occurrence of wildcats was assumed but not confirmed. As this mountain range can function as an important habitat linkage between the Harz Mountains and the Eifel population, we aimed to reveal the current distribution of the species in this area. To detect the elusive wildcat, we first deployed 15 camera traps at game trails in a forest area of 15.000 ha from December 2020 till April 2021. During 1778 camera trap days 100 events of wildcat detection were obtained. These results gave the first indication of a wildcat population in the Teutoburg forest. To confirm these results, a genetic analysis via collected hair samples was conducted in a second step. Lure sticks treated with valerian solution where placed in front of the camera traps. In total, 30 hair samples were collected in January and February 2020. Genetic analysis revealed nine different individuals which all where determined as true wildcats. Despite the proximity to settlements and even camera trap detections of domestic cats, no evidence of hybridization of both species was found. Our results confirm the occurrence of European wildcats in the Teutoburg forest which makes this area an important step stone for the connection of wildcat populations in Germany.

Stressed out? Using stress hormones to quantify differences in habitat quality of fire salamander larvae

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The larval stage can be considered the most critical phase in the life cycle of the European fire salamander (*Salamandra salamandra*). While adults have only few natural predators and live in terrestrial habitats, larvae are living in vulnerable water bodies that can be changed drastically by the weather conditions (e.g. droughts, heavy rainfalls). Additionally, they are facing competition and predation. While predation and density of conspecifics is low in streams and food availability is high, the situation is the opposite in the ponds, which is why ponds are considered the less suitable habitat. However, previous reciprocal transplant experiments demonstrated that larvae in ponds performed better. Abiotic and biotic factors can function as environmental disruptors and influence the development by altering the thyroid hormone level. Stress hormones enhance the sensitivity of the organism to thyroid hormones and can thus have a strong impact on the phenotypic plasticity of an individual and in this way cause fitness consequences. Corticosterone (CORT) is a useful biomarker to assess the condition of amphibians as this “stress hormone” is responsible for homeostasis and growth levels. Moreover, amphibians with a high CORT level are more susceptible for parasitic fungus infections which is particularly important considering the current spread of *Batrachochytrium salamandrivorans*, a chytrid fungus which can cause the extinction of whole populations. The aim of this study is to measure individual water-borne CORT release of pond and stream larvae in a reciprocal transplant experiment (before and after transfer) to understand in which habitat larvae are more stressed. We captured in total 64 larvae at two ponds and two streams in April 2022 in the Kottenforst (Bonn, Germany) and measured baseline CORT release immediately after capturing as well as stress induced CORT afterwards. Then, we transferred the larvae for two weeks in individual enclosures in the same or different habitat and we measured CORT again. We are currently determining the CORT concentrations. We hypothesise that i) larvae from ponds have a higher CORT release than larvae from streams, and that ii) larvae transferred into ponds have a higher CORT release than larvae transferred into streams. Considering the environmental changes through global warming, it is important to understand, which larval habitat is more beneficial and thus should be considered in the future for conservation purposes.

Unilateral adaptation - how trichomes change pollinators' behavior in sexually deceptive flowers

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The close adaptation between the sexually deceptive orchid *Ophrys* and its pollinators offers great potential to study evolutionary processes. *Ophrys* flowers resemble female bees and attract males for pollination in a species-specific manner. Therefore, to understand speciation processes within *Ophrys*, it is important to study pollination-relevant traits of the flower lip (labellum), with which the bees interact during 'pseudocopulations'. So far, knowledge is limited on the intraspecific significance of the labellum's surface structure. In this study, we address the importance of trichome patterns on the labellum, aiming to link genetics, phenotype, and reproductive fitness. Therefore, we studied the expression of genes likely important for trichome development, comparing trichome-rich and trichome-less tissues of the labellum. Additionally, to get detailed insights into trichome-dependent behavior of pollinators during 'pseudocopulations', field experiments were performed in which untreated flowers (control) and flowers after removal of trichomes in different regions (basal, median, and apical) were offered to male pollinators. This revealed that behavioral changes on the basal and apical treated flowers were present. The removal of basal trichomes resulted in a significant decrease of 'pollinia removal' frequency and therefore had a direct impact on plant reproductive fitness. Manipulation of trichome density in different parts of the lip allowed us to shed light on the ecological function of trichomes, and to assess the importance of the spatial patterning. In conclusion, this study contributes to understanding the role of trichomes in pollinator interaction and the genetic basis of trichome development by identifying first candidate genes involved in this process.

Tiny but mighty - Do microbial communities covary with personality?

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Animals exhibit consistent individual differences in various behaviours, called personality. These differences are assumed to be influenced by several parameters, such as environmental or genetic factors. Recent research in laboratory rodents and humans pointed a novel potential source of individual variation in behaviour: the microbial communities residing in the gastrointestinal tracts (gut) of animals can modulate host behaviour via different pathways. However, we still know very little about host-microbiome interaction and its potential impacts on personality. To shed some light on this topic, we will search for connections between the gut microbiome and the differences in the individual behaviour of wild-type zebra finches *Taeniopygia guttata*. To receive some information about individual personality traits we tested zebra finches bi-directionally selected for three personality traits: aggression against a mirror image, exploration in a novel environment and fearlessness measured by the duration of tonic immobility. At the same time of behavioural testing, we collected faecal samples. These samples will be used to characterize the gut microbiota using 16s ribosomal RNA gene sequencing to understand whether certain behavioural traits are associated with individual variation in gut microbiota. Our study will broaden our understanding of the evolutionary consequences of host-microbe interactions.

First data on an assessment of the diversity of silverfish (Zygentoma: Lepismatidae) in the Atacama Desert

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Silverfish are among the most important consumers in deserts ecosystems worldwide. In the Namib, which is well explored regarding silverfish, their diversity is high. The diversity of silverfish in other old deserts e.g., the Atacama Desert is often unknown. In the Atacama Desert, no data on silverfish other than those belonging to the family Maindroniidae are known. Field sampling allowed a first comprehensive phylogenetic analysis of COI sequences and thus also an assessment of the diversity of the Atacama silverfish belonging to the species-rich Lepismatidae. The data suggest that the Atacama Lepismatidae are separated into five clades. Two of these clades also contain worldwide distributed *Ctenolepisma* and *Thermobia*, while the three remaining clades consist of unknown taxa with several species each. These data clearly show that our knowledge of silverfish from this large region of South America is still in its infancy.

3D shape and biomechanical effects of *Daphnia atkinsoni*'s inducible defence, the 'crown of thorns'

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The adaptation of organisms to their environment is one of the most important basic characteristics of life. A particularly spectacular form of this adaptation is phenotypic plasticity. Predator induced defences, a special form of phenotypic plasticity, are well-known to render prey species less susceptible to predators, for example through alterations of shape and behaviour. Based on our current analyses of the inducible defences in *D. atkinsoni*, we can assume that in this species morphological rather than behavioural changes are tailored to unleash their protective potential. Such inducible defences are well-known in many Daphnia species. For example, *D. atkinsoni* has been shown to form so-called "crown of thorns" at the dorsal part of the head capsule in the presence of *Triops cancriformis*, a tadpole shrimp (Notostraca). Despite these alterations are well-known, their function in how they protect *D. atkinsoni* and reduce the predation risk is still unknown. Therefore, we aimed at a holistic analysis of this species defensive strategy. While we did not know behavioural alterations, we focused on the investigation of the shape alteration in detail using confocal microscopy and subsequent 3D modelling. In order to unravel streamline effects of morphology during swimming, we used biomechanical tools, such as streamline simulation. Subsequently, we created a 3D model of Triops-exposed vs. unexposed *D. atkinsoni* and determined the swimming velocities and the angle of attack to reveal the streamline properties of both *D. atkinsoni* morphs. By this we can now draw a holistic picture of the protective mechanism and their mode of action in *D. atkinsoni*. As inducible defences incur costs, our analysis now provides the basis of a comprehensive understanding of costs and trade-offs associated with this defence.

Establishment of an eDNA method to survey the distribution of endangered crayfish species.

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Managing biological diversity requires accurate identification; however, many species are difficult to identify with standard morphological approaches. Conservation sciences rely upon precise species counts to better protect and map organisms. One problem that abounds in this type of research is that surveying endangered species may require the sacrifice of the individuals, which isn't desirable as their study could contribute to their decline. DNA barcoding is a widely used tool and has been shown to successfully supplement morphological identification and can even reveal cryptic species. In this study, we survey the distribution of endangered crayfish species in Texas, USA, and develop a method to survey species indirectly. First, we employed standard COI barcode sequences using crayfish-specific primers (orcoCOI-F/R) on DNA extracted from collected specimens. Second, we employed eDNA ('environmental DNA') to detect crayfish species indirectly from water samples. The concept of eDNA detection relies on the assumption that all organisms release their DNA to a collective pool of DNA in the environment through decomposition, shedding, waste production, etc. In this method, environmental DNA is extracted from water filters and purified. This assay enables us to detect multiple crayfish species simultaneously. In doing this, the individuals of the species could potentially be spared the intrusions of physical surveying methods, and the data gathered could be much more abundant, as the eDNA method can be quicker and cheaper to utilize.

Compass neurons and the real sky

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A number of insects use various features of the natural sky as guiding cues for navigation. Laboratory experiments on desert locusts showed that neurons of the central complex code for polarization patterns of the sky that match particular solar positions and are in line with solar azimuth coding by responses to unpolarized light spots (Zittrell et al. 2020, PNAS 117:25810). This suggests, that locusts can use both cues for spatial orientation when exposed to natural conditions. To test this, we used a hut above the treetops to record extracellularly from units in the central brain of the locust *Schistocerca gregaria* (Forskål, 1775) under laboratory and natural sky conditions. Inside the dark hut, the animals were exposed to polarized blue light from above. After the polarizer was rotated above the stationary animal, the locust was rotated while the polarizer remained stationary. After opening two hatches in the roof the animals were lifted beyond the roof top and passively rotated by 360° under the natural sky. Celestial polarization and spectral properties were recorded with a camera and a spectrometer shortly before and after testing unit responses to the natural sky. The three stimulus conditions could be tested up to three times during recordings lasting one to three hours. Unit responses to artificially polarized blue light had the same axial preference angles when the polarizer or the animal were rotated and thereby provided ambiguous directional information. When rotated under the sky, however, the same units usually responded with circular preference angles, providing unambiguous directional information. The azimuthal preference angles, when exposed to the sky, were shifted by approximately 90° relative to their axial preference angles to polarized light in the hut. The results suggest that units either respond to sun azimuth and/or a matched-filter representation of the sky polarization. This is currently being tested by obscuring the sun or the polarization pattern when the locust is exposed to the sky.

The myology of the crocodile lizard, *Shinisaurus crocodilurus* Ahl, 1930 – Part I: Description of the anterior extremity

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The crocodile lizard, *Shinisaurus crocodilurus* Ahl, 1930, is a rare and endangered squamate occurring in south-east China and north-east Vietnam. It is the only extant representative of the Shinisauridae, which are an early offshoot of the anguimorph clade that includes the earless as well as the true monitor lizards. The overall phylogenetic relationships within anguimorphs remain controversial when morphological and molecular data are compared, especially regarding the placement of the Helodermatidae, and whether they belong to the aforementioned clade or not. The majority of morphological data so far are based on the skeletal system only, whereas information on soft tissues is rather sparse. The present study forms the initial part of a broader approach to anguimorph morphology that will focus on the comparative myology of a number of species, including helodermatids and varanids. This particular subproject deals with the anterior extremity of *S. crocodilurus*. A total of 6 alcohol-preserved specimens (snout-vent length 135–156 mm) were manually dissected for the present study. The specimens were skinned and each individual muscle was isolated by scalpel and spatula until the bone was reached. Consecutive numbers were assigned to each of the resulting muscle bellies, totalling to 105 separate units whose origin and insertion was documented photographically and by drawings. These descriptive data now will serve the basis for a future comparative approach.

An aqueous extract of the brown alga *Eisenia bicyclis* extends lifespan by interfering with the Tor-FoxO axis

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Marine algae serve as food supplements in many countries around the world. Their beneficial effects have attracted the attention of researchers to decipher their mechanism of action and exploit them to enhance the life- and health span. In this study, we elucidate the effects of an aqueous extract of the marine brown alga *Eisenia bicyclis* on life span using *Drosophila melanogaster* as a model. We found that addition of a small amount of *Eisenia* extract increases the life span of female flies by about 40 %. However, the *Eisenia* extract was not beneficial for male flies, showing the extract's life span-extending effect is sex-specific. In flies subjected to a high sugar diet, the *Eisenia* extract enhanced median and maximum life span by more than 30 %. A comprehensive and in-depth mechanistic analysis revealed that these life-prolonging effects depend on functional Tor and FoxO signaling. Based on this, it seems that the life-prolonging effects of *Eisenia* extract depend on a functional Tor/FoxO axis. This study might whet further research on the translational applications of *Eisenia bicyclis* and identifying substances responsible for its effects.

RNAi soaking induces gene knockdown of olfactory ionotropic co-receptors in *Daphnia pulex*

Jana Terbeck; Linda Weiss

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Freshwater crustaceans of the genus *Daphnia* play a central role in freshwater ecosystems. They are the main consumers of phytoplankton and at the same time they serve as an important food source for second-order consumers. *Daphnia* can reduce their predation risk by developing morphological adaptions. For example, *Daphnia pulex* develop neckteeth when exposed to predatory phantom midge larvae of the genus *Chaoborus* (Diptera). These larvae release chemical signaling cues, so-called kairomones, when feeding on their prey. *D. pulex* can sense these cues via a conserved group of ionotropic receptors (IRs). Using micro-injections of double stranded RNAi probes targeting the chemo-co-receptor genes [IR25a] and [IR93a], *Daphnia* lost its senses of smell. However, these microinjections can only be performed in larger sized *Daphnia* species. Micro-injections into small species such as *D. pulex* or *D. cucullata* are significantly more difficult to perform. We therefore here aimed to establish a simplified RNAi delivery method that also enables high-throughput screenings. We therefore soaked *Daphnia pulex* mothers with freshly deposited embryos in the brood pouch in kairomones and ds-RNA probes (1,2 µg/ ml) targeting the [IR25a] and [IR93a]. As controls we used a dsGFP probe. We then measured neckteeth expression in the 2nd juvenile instar. In [IR25a] and [IR93a] ds-RNA soaked specimens we found a significant reduction of neckteeth formation in comparison to the control. These results will be further validated using quantitative PCR. In addition, we aim to identify if there is a distinct developmental period during which the probe has to be delivered. This simplified method of ds-RNAi probe delivery will enable fast and efficient high-throughput screenings of selected target genes involved in manifold molecular mechanisms.

Innervation of pectoral and pelvic fins in the amphibious mudskipper

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Innervation of pectoral fin muscles in actinopterygian fishes originates from conserved sites in the hindbrain and spinal cord (SC), in contrast to the SC-only innervation of forelimbs in tetrapods. A gradual change to the SC-only innervation and an aggregation of pectoral innervating nerves to more rostral SC segments can already be seen in certain sarcopterygian species that share recent common ancestors with tetrapods, e.g. the lungfish (Dipnoi). Among actinopterygians, the amphibious mudskippers (Periophthalmidae) are also on the verge to a terrestrial lifestyle. This is not only reflected by anatomical adaptations to the terrestrial habitat, but also by distinct locomotor behaviors that allow mudskippers efficient locomotion underwater and on land. Mudskippers therefore use coordinated movements of pelvic and pectoral fins, necessitating elaborate interconnectivity of spinal neural networks that innervate the pectoral and pelvic muscles. We used in-vitro backfills of pectoral and pelvic nerves to investigate the distribution of their associated motoneurons (MNs) in the African mudskipper (*P. barbarus*). Combinations of pectoral and pelvic nerves were stained with Dextran-Fluoresceine (3 kDa) and Dextran-Rhodamin (3 kDa). Additionally, neurobiotin was used in some experiments to test for trans-neuronal staining of pre-motoneurons (PMNs). Three nerves innervate the pectoral and two the pelvic fin muscles. Preliminary data suggest that two nerves innervating the pectoral fins originate from the SC, and one nerve originates from the caudal hindbrain, where it splits into occipital nerves 1 and 2. The pelvic muscles are innervated exclusively from SC segments 3 and 4. Therefore, similar to e.g. lungfishes, an aggregation of pectoral innervating nerves to more rostral SC segments took place in mudskippers. Backfills also show that pectoral and pelvic MNs and PMNs are not only located within the SC segments of their specific nerve. Interestingly, some MNs and PMNs also radiate into adjacent SC segments and intermingle with MNs and PMNs associated with different pectoral and pelvic nerves. Trans-neuronal labeling of PMNs could be detected, suggesting gap junctional coupling to the motoneurons. Our findings suggest that in mudskippers, analogous to some sarcopterygian species, a shift of motor networks that control the coordinated movement of pectoral and pelvic appendages occurred. This adapted neuroanatomy presumably facilitates terrestrial locomotion.

Male Guinea baboons are oblivious to their females' whereabouts

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For group-living animals, it is advantageous to track the whereabouts, state, and interactions of groupmates to predict their future behaviour. Yet, there has been considerable debate about whether life in a complex group per se or rather the degree of competition prevalent in a group is the driving force behind such social monitoring. In this study, we investigated whether wild Guinea baboons *Papio papio* keep track of their females' location. Guinea baboons live in a low-competitive nested multi-level society with one-male units consisting of a primary male, between 1-6 females and their young at the base of the society. Females enjoy relatively high spatial freedom; they can roam unimpeded and interact with other group members with rare male interference. Using field playback experiments, we tested whether male Guinea baboons (N=62 trials, N=22 males) keep track of the whereabouts of their unit females. We played back vocalisations of unit females from a location either consistent or inconsistent with the actual position of the female after both animals had separated. In a second experiment we tested whether primary males (N=14) responded more strongly to vocalisation of unit-females vs. non-unit females. In the main experiment, males did respond clearly to the playback, but contrary to our predictions, they did not show any signs of surprise (longer looking duration or shorter latency) when the female's calls were played from a physically impossible location. While males seem to be able to recognize their female by voice, as evidenced by stronger responses to calls from unit females than non-unit females, they apparently lack the ability or motivation to track their females' movements. In contrast, the highly competitive chacma baboons *Papio ursinus* showed high sensitivity to deviations from expected patterns of female locations. Taken together, variation in the degree of competition and control rather than life in a socially complex society appears to drive the allocation of social attention.

Morphology of the female reproductive system of *Rhithropanopeus harrisii*

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The Panopeidae (Decapoda: Brachyura) is a family commonly known as mud-crabs and is native to the Atlantic and eastern Pacific Ocean. Several species are well known as invasive neozoan species in European waters. For example, the North American whitefingered mud crab, *Rhithropanopeus harrisii* (Gould, 1841). In Europe, this invasive species was first discovered 1874 in the Zuider Zee in the Netherlands. Since then, it has become one of the most widely distributed crab species worldwide. To get a better understanding of their successful invasion strategies, the species' reproductive could provide first answers. The outer morphology of the male copulatory organs, the gonopods, were already investigated for taxonomic purposes. In contrast, the inner morphology of the female reproductive system needs to be investigated to understand the interaction between the male gonopods with the female reproductive organs. Therefore, in this study, the morphology of the female reproductive organs of *Rhithropanopeus harrisii* were investigated. A deeper understanding of the structure and function of the reproductive system enables an understanding of the mechanisms of egg fertilization and sperm transport. We used histological and morphological methods to reconstruct a functional 3D model of the female reproductive system to explain the processes from the interaction between the female gonoducts and the male gonopods to the sperm storage modes, and the fertilization mechanisms of the eggs. These results were compared to morphological data of the reproductive systems of other brachyuran crabs. Understanding the functional morphology of the reproductive organs of *Rhithropanopeus harrisii*, the reproductive organs within the Panopeidae can provide important insights into their successful reproduction and invasion.

Behavioral differences in telencephalon ablated cichlids and goldfish

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Fish possess a wide range of cognitive abilities. However, where cognition is being processed in the brain has not been examined in any detail, with the exception of spatial memory and aversive learning. In regards to these, selected forebrain areas (lateral and medial pallium) of ray-finned fishes have been hypothesized to be homologous to the hippocampus and pallial amygdala of land vertebrates. Areas that have not been assessed, but are also likely to be involved in cognitive information processing, are the central, dorsal, and ventral regions of the telencephalon as well as several diencephalic and mesencephalic regions. In this study, the telencephalon of the cichlid *Pseudotropheus zebra* and the goldfish *Carassius auratus* were ablated to determine the telencephalic involvement in regards to object inspection and novel exploratory behavior. Telencephali were removed by suction, using a glass Pasteur pipette connected to a syringe. Fish were monitored pre- and post-operatively using a video tracking system. By comparing pre-operated, operated, and sham operated fish, variations in swimming pattern, usage of shelter, object inspection and tank spatial behavior were found. Telencephalon ablated fish avoided the shelters, the novel objects, and the walls of the tank, preferring to stay in open areas. Exploratory behavior of post-operated fish when presented with a new object was absent or severely reduced. Follow-up research will look at ablation effects of specific areas of the telencephalon and diencephalon.

The effects of multiple stressors on biotic interactions in riverine ecosystems: A case study on predator-mediated shifts on macrozoobenthos behaviour

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Biotic interactions in freshwater ecosystems are frequently modulated via chemical compounds. This is particularly well known for predator-prey interactions, e.g. recognition of fish predators by insects. However, anthropogenic stressors in aquatic ecosystems are suspected to hinder predator recognition. One of the suspected causes is the impairment of the prey physiology or sensory system, resulting in their inability to respond appropriately to predator signals, yet data on the impacts are rare. Therefore, we studied the influence of stressors on these interactions in a stream macroinvertebrate community within a large experimental setup. Using a highly replicated field-mesocosm setup (the so-called ExStream System), we aim to investigate how single and multiple stressors can affect chemical information transfer networks of predator-prey interactions as well as classical food webs in a full factorial design. The study site is a river section of the Boye, a tributary of the Emscher River, which has been fully restored in 2021. It was used to test how the increase and release of anthropogenic stressors i.e. increased salinity, reduced flow velocity and increased temperature influence the biotic community. With these experiments, we want to elucidate the influence of stressors on indirect and direct predation effects on macrozoobenthos by introducing kairomone-enriched water or fish predators into the mesocosms. Macrozoobenthos responses will be related to different stressor combinations and may be influenced by already existing resistances due to the years of stressor exposure history of the Boye communities.

The control of multiscale rhythms in sensitivity and kinetics in pheromone transduction of the hawkmoth *Manduca sexta*

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Hawkmoth males respond to pheromone concentrations over at least 4 log units apart, making the process of pheromone transduction a highly sensitive affair with a very large reaction range. It is still not understood which mechanisms and ion channels are responsible for this astoundingly extended response range. Pheromone transduction cascades in the hawkmoth differ daytime-dependently and are adjusted by stimulus strength via mostly unknown mechanisms. Olfactory receptor neurons (ORNs) innervating long trichoid sensilla of the antennal flagellum display daily rhythms in pheromone sensitivity and kinetics that are orchestrated by circadian oscillations in cAMP concentrations in antiphase to cGMP levels. Furthermore, ORNs are also ultradian oscillators, displaying fast rhythms in spontaneous action potential activity indicative of fast membrane potential oscillations, accompanied by fast Ca²⁺ oscillations. It is not known whether these multiscale rhythms are linked and whether their synchronization plays important functional roles. Since cyclic nucleotide levels as well as Ca²⁺ concentrations are acutely modulated by G-protein coupled octopamine- and neuropeptide receptors, biogenic amines and neuropeptides were suggested to modulate odour responses. Patch clamp experiments of primary cell cultures of hawkmoth ORNs confirmed that antagonistic ion channels with fast kinetics and more negative reversal potentials are activated via cAMP while cGMP rises favoured slow channels with more depolarized reversal potentials, indicative of high intracellular Ca²⁺ levels. Thus, we hypothesized that multiscale endogenous clocks of ORNs control push-pull-mechanisms orchestrating sensitivity and kinetics of pheromone transduction with high cAMP- accompanied by low cGMP levels supporting sensitization. Here, we explore whether and how interdependent daily rhythms in second messenger concentrations affect the kinetics of ORNs of long trichoid sensilla already in absence of pheromone stimuli via control of the membrane potential and, thus, the spontaneous activity. We found that spontaneous action potential activity of ORNs expressed interlinked rhythmicity in the circadian and the ultradian frequency range. In summary, our experiments challenge our hypothesis that linked multiscale rhythms of second messengers modify odour transduction over an extended range of sensitivity and kinetics with posttranscriptional modulation of ORCO as vital component.

Biodiversity assessment of desert nematodes using long-read sequencing

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Nematodes are one of the most species-rich groups among metazoans with 28.000 species described and at least one million expected to occur. They can inhabit soils, tropical regions, deep ocean floors, and even extreme ecosystems such as hyper-arid deserts. In low-humidity environments, some nematode species remain in a state of dormancy, called anhydrobiosis, where metabolism is suspended. The diversity of nematodes in extreme environments is not well understood and studies are impeded by geographical remoteness of these habitats. As morphological characters are insufficient to assess biodiversity, in particular some highly uniform taxa, cost-effective, fast field-based sequencing methods are a promising avenue for studies. Through an initial biodiversity assessment of soil nematodes in the Atacama Desert, the driest non-polar desert in the world, we found nematode populations represented in four different genera first using 18S SSU rRNA as a standard marker gene. We then performed near real-time genetic identification of nematodes from soil samples using Oxford Nanopore long-read sequencing in the laboratory. Through this we obtained draft genome assemblies from few individuals, including an assembly of a sexually reproducing diploid *Panagrolaimus* species isolated from soil in the Atacama Desert. These show good BUSCO completeness, comparable to publicly available assemblies, thus enabling robust phylogenomic placement of uncultured species. We will now develop a fast-on-site sequencing workflow to enable genetic direct identification of desert nematodes from remote habitats implementing portable laboratory equipment. This method can in the future be used to identify and study soil and parasitic nematodes that serve as bioindicators of disturbance and instability in the environment.

Sex-specific effects of inbreeding on dynamic color-ornament expression in the cichlid fish *Pelvicachromis taeniatus*

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Color expression is highly variable in animals. In fishes, rapid color change is used in multiple contexts, e.g., in camouflage or communication, and is affected by various factors, e.g., stress. *Pelvicachromis taeniatus* is a cichlid fish from West Africa with sexual dichromatism and both sexes being brightly colored and flexible in ornament expression. Males show a carotenoid-based ventral coloration whereas the violet body coloration of females consists of structural and carotenoid-based colors. In the present study, digital photo analyses were used to compare the dynamic color expression of inbred and outbred males and females following a stress exposure. The chromaticity and the color patch size (relative colored area at the abdomen) were determined before and after stress by handling, and the change in coloration was analyzed. Additionally, the coefficients of variation within family groups for the chromaticity (CVchromaticity) and color patch size (CVarea) were calculated. Chromaticity as well as the extent of coloration increased significantly following stress exposure. The change in chromaticity was not significantly different between in- and outbred individuals in females and males. Inbred males showed more intense yellow coloration than outbred males. Independent from inbreeding, the CVchromaticity and the CVarea decreased after the stress exposure. The change in CVarea of females and males differed between in- and outbred individuals. In females, the decrease was significantly stronger in inbred individuals, whereas in males, the decrease was stronger in the outbred group. The results show that short-term stress can increase coloration, potentially advertising individual's stress tolerance. Furthermore, this study shows positive inbreeding effects on a sexually selected trait.

Internal states are co-involved in the determination of open field behaviour in Drosophila

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The open field test (OFT) is often used in rodents for scoring anxiety and depression levels. In the OFT, fruit flies show wall-following behavior (WAFO) similar to rodents, influenced by homolog anxiety genes. Therefore, the OFT in *Drosophila* has been suggested as a "new neurogenetic tool for anxiety research". However, it is questionable to which extent insects are able to perceive emotions such as fear. To better characterise the influence of emotion-like states of the OFT behaviour, we measured both WAFO and total walking distance (TWD) in the OFT in WT flies under different stress-related conditions or treatments. Our results measured in several lab- and nature-derived WT strains show a positive correlation between WAFO and TWD, while a negative correlation is found in rodents. Besides, nature-derived flies showed significantly higher WAFO and TWD values than lab strains. A comparison between OFT and DAM data suggest that WAFO and TWD are not correlated with the spontaneous activity level. The locomotor activity in our small-diameter OFT arena is unlike the spontaneous activity reported for larger diameter arenas or the DAM system. Interestingly, we observed an uncoupling of WAFO and TWD under stressful social and physiological conditions. Furthermore, there is no circadian effect in the OFT behaviour since neither WAFO, nor TWD is changed in a daily manner. Our results show that the OFT behaviour of flies is influenced by their defensive level, and confirm and strengthen the role of internal "emotion-like" states, which is also supported by changes in WAFO upon manipulation of serotonin and peptidergic signalling. Our data improves the understanding of the complex open field behaviour of flies and show that the OFT analysis reports escape-driven defensive levels that are influenced not only by external sensing, but also by internal states.

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An interactive biomimetic fish robot helps to understand the rules of information transfer in groups of weakly electric fish

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Animals that live in social groups exchange information via various communication channels in order to recognize and react to conspecifics. The ability of individuals within a group to respond and adapt to the actions of their social partners often allows them to come to joint decisions and engage in coordinated behaviors. In mormyrid weakly electric fish, individuals continuously emit and perceive pulse-type electric organ discharges (EOD) to establish highly complex social group dynamics. This electro-communication allows mormyrids to exchange identity information based on the waveform of their EOD, as well as contextual information through the modification of their inter-discharge intervals (IDI). Our previous studies have shown that shoaling mormyrids frequently engage in episodes of interactive electric signaling by synchronizing their EODs to each other and also interact with artificial signal sources like an EOD-emitting fish robot. However, it is not fully known which social cues these signals convey, what they mean, and which behaviors they evoke in other fish. Here, we investigate the basic processes that facilitate social interactions in shoals of weakly electric fish by developing an interactive biomimetic electric fish robot ("ElectroFish") that serves as a communication partner. Because during EOD synchronization two fish respond to each other within a short time window of 20-30ms, we developed an automated, on-line EOD localization system using neural networks to allow for real-time identification of the EOD sender within a group. For subsequent directed responses of the robot such as interactive locomotion and EOD synchronization, we also developed a response system that integrates the electric signaling properties into the robot control software. By designing this fully interactive robot, which operates in closed-loop both electrically and locomotorily, we have full control over the cues we inject into the social system. Thus, we are able to investigate effects of ensuing locomotor and/or electrical interactions on behavior and to understand the rules of information transfer between individuals in social groups of weakly electric fish.

Lifestyle reconstruction of two extinct species of squirrels (Mammalia: Sciuridae) using femoral shape: further evidence for the arboreal origin of squirrels?

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Correlations between morphology and lifestyle of extant taxa are useful for the prediction of lifestyles of extinct relatives. In the case of squirrels (Sciuridae), the oldest known and also nearly complete fossil of the genus *Douglassciurus* from the early Oligocene of Wyoming, North America displays many characters found in extant tree squirrels. Two extinct species from the middle Oligocene and early Miocene of France (*Palaeosciurus goti* and *P. feignouxii*, respectively), however, were previously reconstructed as terrestrial. We here re-evaluate their most likely lifestyle based on femoral shape. We sampled six well-preserved specimens of *P. feignouxii*, the single existing specimen of *P. goti*, as well as 180 extant species of squirrel-related rodents (Sciromorpha: Sciuridae, Gliridae, and Aplodontiidae) with one specimen each. From three extant species, we sampled 20 specimens to determine intraspecific variability and judge the confidence of lifestyle predictions based only on a few fossil specimens. Applying a geometric morphometric approach, we found *P. goti* to cluster within its extant arboreal relatives, whereas *P. feignouxii* clustered in a shape area with overlapping arboreal and fossorial/terrestrial species. We also used various univariate measurements to evaluate which bivariate subset best predicts lifestyles. Combining femoral length with another variable was always essential, but the choice of the second variable (measurements of the head, condyles, patella, midshaft etc.) did not affect the accuracy of our lifestyle discriminations. All these combinations confirmed the geometric morphometric results. Our findings provide further evidence for arboreality already being the most common lifestyle among the earliest squirrels, since the older fossil species (*P. goti*) could be confidently predicted as arboreal. In contrast, *P. feignouxii* might have exhibited a terrestrial lifestyle, which shows that squirrels quickly evolved different lifestyles that are still observed among extant taxa. Our bivariate linear measurements are useful for future predictions of lifestyles from new fossil specimens with incomplete femora. Yet, in such cases, femoral length appears to be a necessary feature to make reliable predictions.

The neuropeptide pigment-dispersing factor (PDF) controls seasonal adaptation to changing light periods in the equatorial cockroach *Rhyparobia maderae*

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Due to the earth's rotation around its axis and its rotation around the sun, organisms are exposed to a day-night cycle of about 24 h as well as changing photoperiods at a cycle of a year. To anticipate favourable times for rest and specific activities, organisms evolved endogenous circadian clocks that generate a rhythm of about 24 hrs. In addition, internal clocks allow adaptations to the changing length of daylight during the year. The Madeira cockroach *Rhyparobia maderae* is an established model of chronobiology. Transplantation experiments identified the accessory medulla (AME) in the brain's optic lobes as the circadian clock that controls rest-activity cycles. Immuncytochemical and behavioral studies showed that AME clock neurons (PDFAMEs) expressing the neuropeptide pigment-dispersing factor (PDF) orchestrate sleep wake cycles of the cockroach. We want to know whether/how an equatorial cockroach that evolved under equinox conditions does adjust to acute/permanent changes in photoperiod with its PDF expressing circadian clock. Apparently, adaptation to changes in photoperiod involve two circadian oscillator circuits: the morning (M) oscillator locked to dawn and the evening (E) oscillator tracking dusk. While in the fruitfly *Drosophila melanogaster* PDF expressing clock cells are only part of the M clock, distinct PDF clock cells of *R. maderae* appear to participate in M- and E clocks (Gestrich et al., 2018). Here, we describe changes in mRNA levels of PDF precursor in cockroaches either raised in- or acutely transferred to different photoperiods. Both groups showed elevated mRNA levels of PDF precursor but not PDF receptor under long-day conditions (LD 18:6), and decreased levels in short-day conditions (LD 6:18) compared to cockroaches from LD 12:12. Additionally, we found photoperiod-dependently increased or decreased numbers of PDF-immunoreactive PDFAMEs. A posterior group of PDFAMEs increased in long-day conditions and an anterior group decreased under short-day conditions. In conclusion, even the equatorial Madeira cockroach adapts to different photoperiods either when raised at a specific photoperiod during ontogeny, or when acutely transferred to a new photoperiod as adult. Interestingly, distinct PDF clock neurons allow for light-dependent adjustment of sleep-wake cycles.

The role of the Andean uplift in the diversification of the Elenophorini (Coleoptera: Tenebrionidae) in South America.

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Darkling beetles of the tribe Elenophorini are composed only by three genera: Leptoderis from Europe and Psammetichus and Megelenophorus from South America. This classification and distribution suppose an ancient origin of this tribe. However, Leptoderis and Megelenophorus are only represented by a single species each, while Psammetichus have many. This unbalanced diversification seems to be linked with the geo-climatological events in the past, which affected more to the Psammetichus which is distributed only on the western slope of the Andes of Peru and Chile. In order to know the spatio-temporal diversification of the Elenophorini of South America, we reconstructed a phylogenetic tree with a set of mitochondrial and nuclear genes and then we estimate the divergences times. The data suggest an early diversification in Elenophorini of South America and the Andes uplift would be the event that forced the separation of the main clades. However, diversification within would be linked with the climate changes of the Plio-Pleistocene.