

ABSTRACT BOOKLET



September 4th-8th 2023

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Voting sheets are provided in the welcome bag
Votes to be handed in the respective voting boxes at the
registration desk
Deadline: Friday by 10:00

VOTE FOR THE BEST POSTER!

REQUIREMENTS TO BE AN AWARDEE:

1. MAKE AN EXCELLENT POSTER
2. PITCH A POSTER PERFECTLY
- (OPTIONAL)
3. NETWORK WITH POSTER AUDIENCE

Student Award

DZG 2023 KASSEL DFG Deutsche Forschungsgemeinschaft multiscale clocks UNIKASSEL UNIVERSITÄT

VOTE FOR THE BEST STUDENT TALK!

REQUIREMENTS TO BE AN AWARDEE:

1. PRESENT AN EXCEPTIONAL TALK
2. NETWORK WITH AUDIENCE

Student Award

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Moderation: Birgit Gemeinholzer (Botany Kassel)

Arten adieu?

Christoph Scherber

Leibniz Institute Bonn

Auch vor unserer Haustüre findet Artensterben/Artverlust statt. Verlieren wir "nur" die Spezialisten aber die anpassungsfähigen Generalisten werden überleben? Wer kann wie dazu beitragen, das Artensterben vor der Haustüre zu stoppen und in welchen thematischen Bereichen gibt es noch Forschungsbedarf? Insbesondere wollen wir der Frage nachgehen, wie Nachhaltigkeit und Biodiversität zusammenhängen und welche Forschung das *Kassel Institute for Sustainability* bzw. das Leibniz Institut zur Analyse des Biodiversitätswandels betreibt, bzw. welche Forschung und welche weitere Unterstützung zukünftig nötig sein wird, um dem Artenverlust entgegenzuwirken.

Panel discussion:

Discussion partners: Christoph Scherber (Leibniz Institute Bonn), Andreas Christian Braun (Center of Sustainability Kassel)

Moderation: Prof. Dr. Monika Stengl (Neuroethology, Sensory Physiology)

Discussion partners: Dr. Violetta Pilorz (Chronobiology), Prof. Dr. Olaf Stursberg (Control and System Theory), Olaf Pyras (Musician, Artist)

Organisms' physiology and behavior are kept in balance, maintained by transitions between homeostatic set points. They form positive feedforward and negative feedback loops, reminiscent of the structure of an endogenous clock generating biological time. Endogenous clocks are oscillators with a sensor that detects and synchronizes with environmental or endogenous cycles. Such rhythms that the clocks entrain to are zeitgebers, like the daily light-dark cycle, or social cues like pheromone pulses released by conspecific mates. We want to discuss how coupling of endogenous clocks determines the expression of biological time, indicators of which we measure as scientists from different research fields, from physiology to evolutionary biology. Furthermore, we want to discuss time perception also in humans and will listen to acoustic examples revealing how our brain tunes into rhythms. Answering questions of the audience, at the round table are sitting Dr. Violetta Pilorz (Chronobiology), Prof. Dr. Olaf Stursberg (Control and System Theory), Olaf Pyras (Musician, Artist), and Prof. Dr. Monika Stengl (Neuroethology, Sensory Physiology).

Whole brain connectomics

Albert Cardona

Medical Research Council Laboratory for Molecular Biology (MRC LMB), Neurobiology Division, UK & Department of Physiology, Development and Neuroscience (PDN), Cambridge University, UK.

I will present our past work analyzing neural circuits in the brain of the larval *Drosophila* – from sensory inputs to descending neurons that control behaviour– and introduce ongoing microscopy and software engineering work in the lab towards imaging and mapping the connectomes of other organisms such as a small cephalopod.

Where big and small data meet

Julia Fischer

Cognitive Ethology Laboratory and Department for Primate Cognition, German Primate Center and University of Göttingen

In the field of nonhuman primate behavior and cognition, the worlds of big and small data meet. New experimental platforms simultaneously record high-resolution video, behavioral, and physiological data synchronously for multiple agents. The amount of data, their accumulation rate and the presence of qualitatively different data streams pose a big data challenge. At the same time, studies in comparative cognition or behavior typically involve few subjects, ranging from a handful to several dozen in nonhuman primate groups, but never meeting the criteria for sufficient power now required in psychological studies in humans. In addition, cognitive experiments are typically limited to a few responses per individual, and observations of behavior in the wild are also relatively sparse. How do we deal with such small data in the face of the replication crisis? One answer are large consortia, like the “ManyPrimates” Initiative, but this approach comes with its own limitations. Irrespective of the drawbacks of small data, their power is that they are collected with a specific goal in mind. When several of such small data studies are collated, we are able to build up cumulative evidence for the cognitive mechanisms and behavioral strategies primates employ to navigate their social and physical environments.

Uncovering the nature of a massive adaptive radiation of cichlid fishes

Walter Salzburger

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walter.salzburger@unibas.ch

The evolutionary phenomenon of adaptive radiation is responsible for much of the ecological and morphological diversity of life on Earth. To uncover the drivers and dynamics of one of the largest extant adaptive radiations, the cichlid fishes of African Lake Tanganyika, we have sequenced a male and female genome of virtually all of the approximately 240 cichlid species endemic to this lake, quantified their morphology on the basis of 2D and 3D x-ray imaging technologies, assessed their ecology using stable isotope signatures, and produced more than 2,000 transcription profiles. Here, I discuss the challenges associated with the collection, management and integrative analyses of this wealth of data, and report some of the key insights into the nature of adaptive radiation that were only possible through such a large-scale operation.

There is an increasing tendency among philosophers, biomedical scientists, gender theorists and other social scientists to promote a relativist view on the biological sex. The proponents argue that the concept of two sexes is too simplistic and that sex is a graded spectrum rather than a binary variable. Leading science journals have been adopting this relativist view, thereby rejecting the well-established concept of biological sex and opposing established fundamental biological facts. Last summer the Humboldt University decided to cancel a public talk on the biological sex because of security concerns. The DZG fully endorses the aim to create an inclusive environment for women, gender-diverse people as well as different ethnicities, but does this require a new definition of the biological sex? In this forum, we would like to discuss the concept of the biological sex from a zoological point of view and its relationship to societal concepts of gender in humans.

Moderation: Gabriele Uhl (Greifswald)

Gender identity is a continuous trait

Impulse Lecture by Diethard Tautz (Plön)

At first glance, the universal occurrence of binary genders appears to be one of the cornerstones of biological knowledge. Male individuals produce sperm, female individuals produce eggs. But does this seemingly clear-cut starting point justify that individuals can only be represented by two sex categories? Individuals arise through a cascade of developmental biological processes. One of these processes controls the development of germ cells and reproductive organs. Other processes control the shaping of the body, i.e., the formation of secondary sexual characteristics. And another process controls the development of the social brain, i.e., the formation of personal preferences and behaviors. Only the very first decision for male or female is usually binary, because it usually depends on the segregation of two chromosomes forming either XX or XY combinations. All other developmental decisions are controlled by polygenic processes, resulting in variability between individuals. Polygenic processes affecting gender identity lead to bimodal distributions, with two means for a given trait and the possibility of overlap. In the case of height, this can be easily illustrated in humans - on average, women are slightly shorter than men, but there are also many men who are shorter than many women and vice versa. The development of overall gender identity involves many individual traits and will therefore be multidimensional, so that in sum they can be viewed as a more or less continuous distribution.

Biological sex is binary. Period!

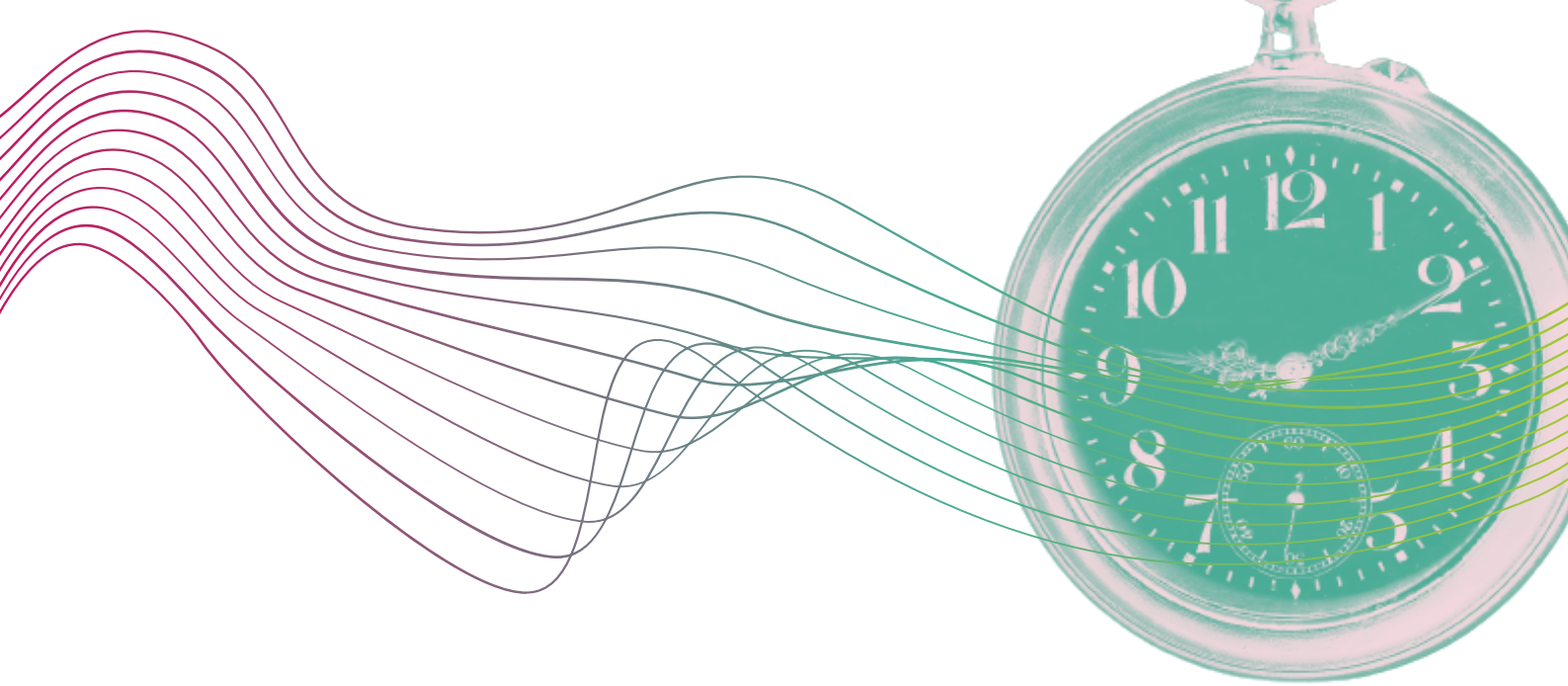
Impulse Lecture by Wolfgang Goymann (Seewiesen)

In her book *Evolution's rainbow* Joan Roughgarden put it in a nutshell: “[...]’male’ means making small gametes, and ‘female’ means making large gametes. Period!”. Proponents of a relativist view on the biological sex entirely miss the point by thinking that a new relativist definition of biological sex would help to foster an inclusive environment for women and gender-diverse people. This attitude rejects the well-established biological fact that the majority of multicellular organisms follow exactly two distinct evolutionary strategies to produce offspring, a female one and a male one.

The relativist view seems to be motivated by a naturalistic fallacy (the mistake of a moral judgement based on natural properties), or an appeal-to-nature argument (proposing that something is good because it is natural), thereby overlooking that ‘being natural’ is irrelevant for ethics. Otherwise, natural phenomena such as infanticide or siblicide could be used to formulate ethical guidelines.

The fundamental definition of biological sex is gamete size, and gamete size is binary. This should not be confused with the process of sexual differentiation, sex-associated genotypes and phenotypes, or with sex roles, all of which can be overlapping, multidimensional and flexible in both females and males.

The relativist view confuses gender – a social concept only referring to humans – and sex, which is a biological concept referring to millions of sexually reproducing species including humans. Moreover, it is anthropocentric to impose human identity concepts on millions of other species (most of which may not have the mental capacities to philosophize about gender). There is a red line that separates humans with their unique combination of sex and gender from non-human animals which only have two distinct sexes – which can either be expressed in the same or in different individuals at the same or at different times. The binary concept of biological sex remains central to understanding the diversity of life and evolutionary strategies of reproduction. It is also crucial for those interested in a profound understanding of the nature of gender in humans.



RESEARCH TRAINING GROUP - MULTISCALE CLOCKS



Unraveling Cellular Dynamics in *Seminavis robusta*: Exploring Extracellular pH Oscillations, and Circadian Modulation of Lipid Droplets

Giagkos-Ion Chlomoudis, apl. Prof. Dr. Thomas Fuhrmann-Lieker

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34132 Kassel

Seminavis robusta (D. B. Danielidis & D. G. Mann, 2002) is a marine benthic diatom species. Diatoms are contributing to approximately 40% of net marine primary production and around 20% of Earth's annual primary production. Our objective is to unravel the intricate molecular clock control underlying the unique diatom life cycles, exploring questions surrounding cell proliferation regulation, photoperiodism, and intercellular communication. Experiments centered on extracellular pH in *S. robusta*, and Nile Red staining for lipid droplet visualization. Our findings will enhance our comprehension of diatom responses to changing light conditions, shaping their interactions within marine ecosystems.

Long-lasting relaxations in the diatom generation clock

Nico Kubetschek¹, Jonas Ziebarth¹, Roland Klassen², Werner Seiler³, Thomas Fuhrmann-Lieker¹

¹Physical Chemistry of Nanomaterials, ²Microbiology, ³Algorithmic Algebra and Discrete Mathematics, Faculty of Mathematics and Natural Sciences, University of Kassel

The population dynamics of diatoms (Bacillariophyta) has a major impact on the world-wide carbon dioxide assimilation. It is based on a unique proliferation mechanism including a step-wise diminution by asymmetric cell division and restoring of the cell size via auxospore formation. We investigate the life cycle analytically by a linear algebra approach and show the conditions for the occurrence of oscillations in the population state, defined by cell number and size distribution. With computer simulations it is shown that the assumption of a limited lifespan for a newly formed cell can slow down the decay of infraannual oscillations down to millions of generations, explaining the occurrence of such a long-period clock.

How does tRNA modification couple the cell division and nuclear division cycles?

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Modification of tRNA is essential for functions such as reading frame maintenance, aminoacylation and tRNA stability, and a lack of modification can result in growth defects in yeast and human pathologies. Our research focuses on Trm5, a tRNA methyltransferase. In yeast *trm5* mutants there is an uncoupling between the cell division and nuclear division cycles; however, the mechanism through which this happens remains unknown. Our aim is to study the *trm5* phenotype and determine the cause behind the uncoupled cycles.

Phosphorylation of Elp1 and its effect on Elongator function

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In yeast, the 1349 amino acid long Elongator subunit 1 (Elp1) is a phosphoprotein and has tRNA binding activity. The C-terminal domain (CTD) has conserved phosphate acceptor sites and a positively charged basic region. Both domains are required for tRNA carboxymethylation by Elongator, a multiprotein complex important for life in higher eukaryotes. The mechanism with which Elongator activity could be modulated by attachment of phosphate groups remains unknown. In the recent past, additional phosphorylation sites in the CTD of Elp1 have been identified through phosphoproteomic studies. Here we investigated through phospho-mimetic and phospho-ablative amino acid substitution the potential influence of phosphorylation on substrate binding. So far, our data suggest a link between timely orchestrated phosphorylation and tRNA binding.

Linking the cell cycle to membrane oscillations in *Drosophila* syncytial cleavage division cycles

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In *Drosophila melanogaster*, as in other insects, the first cell divisions during embryonic development occur without cytokinesis. At the beginning of the 10th division, the embryo enters the syncytial blastoderm stage, where most nuclei are located at the periphery of the embryo and continue to divide. To avoid interference of dividing nuclei, actin-rich membrane furrows move in between the spindles at the beginning of each prophase and retract during each telophase. The link between the cell cycle and cortical actin and membrane oscillations is unknown. The localization of actin regulatory proteins is coupled with membrane transport through the recycling endosome (RE). RE transport requires Rab11, a small GTPase and Nuclear Fallout (Nuf), a Rab11 effector and adaptor protein to the Dynein microtubule motor. This transport mechanism is responsible for membrane growth during cellularization at cycle 14. We found that the gene *drop out* (*dop*) encoding the single homologue of human MAST kinases, is required for this membrane growth. In a quantitative proteomic approach, we identified the serine 401 residue of dynein-light intermediate chain (Dlic) as potential substrate of Dop. Overexpression of Rab11, Nuf or phosphomimetic variants of Dlic suppressed membrane growth defects in *dop* mutant embryos. This led us to a model in which the phosphorylation of Dlic-Ser401 promotes its interaction with Nuf to control Rab11 dependent transport. This phosphorylation may also regulate Nuf-dependent transport of actin activators during syncytial divisions. To test this possibility, a phosphoablative version of Dlic was overexpressed, which resulted in defective syncytial divisions. However, these divisions exhibited phenotypes resembling Nuf-related defects and spindle assembly checkpoint (SAC) abnormalities. Thus, the phosphorylation of Ser401 might not only be important for Nuf-dependent transport, but also for SAC silencing. We propose that the phosphorylation of Dlic is involved in linking the cell cycle with furrow formation during syncytial divisions.

Investigating the role Protein-Phosphatase 2A (PP2A) as a potential link between membrane oscillations and the cell cycle in *Drosophila* embryos

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The cell cycle in *Drosophila melanogaster* early embryos is a great model to study cell-cycle dependent signaling that ensure correct embryogenesis. In the first 13 cell cycles the nuclei are dividing without a following cytokinesis, which results in a multinucleated embryo without dividing cell membranes. In cell cycle 14 membranes are formed in a process called cellularization and a transition of maternal to zygotic transcriptional requirements takes place. Apart from the transition from maternal to zygotic transcripts, changes also occur in the cytoskeleton, to support membrane formation and establishment of cell polarity. The regulation of these processes are mostly unknown but possible contributors might be the protein phosphatase 2A (PP2A) and the protein kinase Drop out (Dop). PP2A plays an important role in the regulation of the syncytial cell cycles. Dop is the only homologue of human MAST kinases and it is known to play an important role during cellularization by phosphorylating the dynein light intermediate chain. Mass-spectrometry analyses revealed that in embryo lysates all three subunits of PP2A bind to Dop, suggesting that the proteins regulate each other. The aim of this project is to establish the interaction between different subunits of PP2A with Dop first using HEK-293T cells, by co-immunoprecipitation and fluorescence microscopy.

Study of major components of the biological clock and circadian behavior in representatives of Tardigrada (water bears)

Bariş Can Ülkü, Lars Hering, Soumi Dutta, Niklas Metzendorf & Georg Mayer

Department of Zoology, University of Kassel, Germany

The water bear *Hypsibius exemplaris* (Eutardigrada) is a microscopic freshwater invertebrate and a member of Panarthropoda, to which besides tardigrades onychophorans (velvet worms) and arthropods belong. Our extensive genomic and transcriptomic searches revealed that this species has a highly reduced set of genes that are typically involved in the circadian clock of other invertebrates. This set includes the genes *cycle*, *timeout*, *vrillie*, *par-domain-protein 1*, and *clockwork orange*, whereas other essential components of the clock, such as period and both genes encoding the light-sensitive and light-insensitive cryptochromes are missing. To clarify whether or not *H. exemplaris* shows circadian rhythmicity – despite the lack of these essential components – we have established a behavioral assay under artificial day/night conditions. The activity of the animals is recorded with an infrared camera and their movements are tracked and analyzed using machine learning software. Once the results have become available for the eutardigrade *H. exemplaris*, we will focus on components of the circadian clock and time-dependent activity in the heterotardigrade *Echiniscus testudo*, as this species does possess at least the light-sensitive cryptochrome. A comparison between the two distantly related species of water bears will provide insights into the ancestral organization, function, and evolution of biological clocks in Tardigrada and Panarthropoda.

Circadian neuropeptidomics for the analysis of coupling factors controlling multiscale behavioral rhythms in *Drosophila melanogaster*

Deepika Bais, Anna-Sophie Kügler, Anna C. Schneider, Susanne Neupert

University of Kassel

Circadian rhythms allow animals to synchronize behavioral and physiological processes to the environment. These rhythms are produced by core clock neurons in the nervous system that generate and transmit time-of-day signals to downstream tissues, driving overt rhythms. These cells drive and time the major activity peaks at dusk and dawn. The fruit fly *Drosophila melanogaster* uses the active phase, for example, for feeding. This behavior is regulated by signalling molecules such as neuropeptides. To identify potential peptidergic candidates involved in the regulation of feeding behavior at dusk and dawn, we used MALDI-TOF mass spectrometry (MS) and Q-Exactive Orbitrap MS to investigate the circadian neuropeptidome of L3 *Drosophila* larvae under fed and starved conditions. To identify potential neuropeptides regulating feeding behavior and overall activity at different times of the day, flies were treated under fed and starved conditions (24 and 48 hours). Their activities were recorded for 20 minutes before the end of the starvation period, respectively. Statistical comparison of the resulting mass spectra revealed semi-qualitative changes in the ion signal intensities to products of eight neuropeptide genes (sifamide, sNPF, extended fmrfamide, hugin-pk, calcitonin-like diuretic hormone-31, allatostatin-C, corticotropin releasing factor-like diuretic hormone-44, kinin). Behavioral analysis showed differences between the fed and the starved conditions, depending on the time of the day. Based on the semi-quantified results, we started with single cell analysis by MALDI TOF MS using different gfp-GAL4 constructs for cell identification and manipulation. The results of our study provide necessary input for future measurements up to single cell level to study the dynamics in up- and downregulation of neuropeptides underlying mechanisms regulating feeding.

Discovery and functional characterization of RYamide signaling in *Rhyparobia maderae*

Sohail H. Shoaib, Huleg Zolmon, Miguel Theisen, Monika Stengl, Susanne Neupert

University of Kassel

Transplantation studies located the circadian clock of the Madera cockroach *Rhyparobia (Leucophaea) maderae* to the accessory medulla (AME), ventromedially to the medulla of the brain's optic lobes. The AME receives photic entrainment from compound eye photoreceptors and orchestrates circadian rhythms in physiology and behavior synchronized to the external 24h light-dark cycles. About 240 neurons innervate the AME which are abundant of partly co-localized neuropeptides. The best studied neuropeptide of the insect circadian clock is pigment-dispersing factor that controls rest-activity cycles. However, the identity and functions of most other neuropeptides of the circadian clock are not known. In this study, we identified three novel neuropeptides which are encoded on the ry-amide gene in *R. maderae* whose precise expression and function have not yet been determined. We identified the ry-amide gene by transcriptome analysis of the CNS and predicted three RYamides: RYa-1 (pQQFYPPGGRY-NH₂), RYa-2 (GSSTFWSGSRY-NH₂), and RYa-3 (NDRFFIGSRY-NH₂). By applying direct tissue profiling using MALDI-TOF mass spectrometry (MS) and Orbitrap MS of the AME, we obtained all predicted RYamides. Using a polyclonal antiserum which recognizes the C-terminal sequence RY, we described its spatial distribution of RYamide in the clock. For functional analysis within the clock system, we applied quantitative immunocytochemistry on whole mount brains and Ca²⁺ imaging experiments using synthetic RY-1 and RY-2 on primary AME cell cultures at different Zeitgeber times. Results show that RYamides are highly expressed in the AME at the beginning of the day (ZT1) while on mid-day (ZT6) and early night (ZT12) expression levels are low. Previous studies of RYamide in *Drosophila melanogaster* revealed RYamide expression in the gut neurons, suggesting that RYamides might regulate cyclical feeding. Therefore, quantitative immunocytochemistry and qPCR experiments of the peripheral nervous system of satiated or food-deprived cockroaches were performed to investigate transcription levels and peptide levels of RYamides.

Diamond-based platforms for biochemical measurements of time-resolved clock cell signaling in response to external zeitgebers and coupling factors

**Rezvaneh Ghasemitabesh¹, Daniel Merker¹, Daniela Bertinetti², Ahmadreza Alidousti-Shahraki³,
Elfriede Friedmann³, Friedrich W. Herberg² and Cyril Popov¹**

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In the last decades, there has been an increasing interest in biological applications of ultrananocrystalline diamond (UNCD) films since they preserve most of the exceptional diamond properties and combine them with a uniform smooth surface that creates the possibility of planting of cells and attachment of biomolecules. In this study the modified and nanostructured UNCD surface will be used as a bio-sensor platform for detection of biomolecules (neuropeptides such as pigment dispersing factor (PDF) or neurotransmitters) from clock cells of model organisms e.g. cockroach and tardigrade. To achieve an effective biosensor, immobilization of proteins on differently modified UNCD is applied with basically two strategies: covalent and non-covalent binding of the biomolecules to the surface. The success of the immobilization and the different preparation strategies is currently validated with fluorescent studies. The non-covalent binding of green fluorescent protein (GFP) with various concentrations on nanostructured UNCD with different terminations (O-, H- and F-termination) is measured using a microplate reader. The evaluation of the average fluorescence intensity of GFP coupled to the UNCD demonstrated that with decreasing the GFP concentration, the fluorescence is also decreasing. In addition, the UNCD samples treated with oxygen plasma show the highest fluorescence intensity since they have strongly hydrophilic properties, whereas the hydrophobic samples treated with hydrogen and fluorine plasma show lower fluorescence. Then, the data obtained from measurements is modeled mathematically using mass-action kinetics for second order reversible chemical reactions. As preliminary work, the covalent immobilization of some nanobodies against GFP is investigated by photochemical grafting of ω -alkene (TFAAD) as linker on hydrogen terminated UNCD and subsequent reaction with glutaraldehyde on structured surfaces. The final goal will be immobilization of PDF-binding proteins for capturing PDF released from clock cells and fluorescently detected with reporter complex composed of binding protein and fluorophore. This will be the foundation for the development of a UNCD-based biosensor for time-resolved molecule detection.

Tuning to the rhythms: pheromone-sensitive olfactory receptor neurons of the hawkmoth *Manduca sexta*

Aditi Vijayan, Katrin Schröder, Mauro Ariel Forlino, Anna C. Schneider, Martin Garcia, Monika Stengl

University of Kassel

Sensory neurons are tuned to detect and predict various environmental rhythms as an elementary advantage for the animal's survival. We examine and model the molecular mechanisms that allow pheromone-sensitive olfactory receptor neurons (ORNs) of the nocturnal hawkmoth *Manduca sexta* to entrain to oscillations in pheromone presence at circadian and ultradian time scales. For mating, female hawkmoths release pheromone pulses at ultradian frequencies during the night to attract their conspecific mates. The frequency of pheromone pulses encodes the female's location. Thus, for successful reproduction males and females need be active at the same time of day. Insect ORNs are endogenous circadian clock neurons that comprise a circadian clockwork in the nucleus based on transcriptional translational feedback loops (TTFLs). These clocks regulate daily sleep wake cycles and allow for synchronization of male and female behavior. However, the male's pheromone sensitive ORNs are additionally required to resolve ultradian frequencies of pheromone signals for female location. It is not known whether/how the TTFL-clock controls circadian and ultradian rhythms in sensitivity and temporal resolution of ORNs during the day.

We used in-vivo electrophysiological (tip-) recordings from long trichoid sensillae of male hawkmoths to characterize the two pheromone-sensitive ORNs. Rhythms in spontaneous activity with spikes differently distributed within or between bursts indicated at least two ultradian frequencies of membrane potential oscillations. It is not known whether both ultradian membrane potential rhythms are linked and are controlled via the circadian TTFL clockwork in ORNs.

We found that spontaneous spike activity of ORNs expressed interlinked rhythmicity in circadian and both ultradian frequency ranges. Subsequently, we employed pharmacology and computational modelling to examine the role of the HCN-type pacemaker channel and of ORCO, the olfactory receptor coreceptor for control of spontaneous activity. With these studies we attempt to decipher the ORNs' temporal encoding.

Identification of core hub genes via transcriptome sequencing analysis of clock-controlled pheromone transduction in *Manduca sexta*

Yajun Chang, Huleg Zolmon, Monika Stengl

University of Kassel

Insect pheromone transduction is under strict circadian clock control. We study pheromone transduction and its regulation via endogenous clocks in the hawkmoth *Manduca sexta*. While it is generally assumed that insect olfactory receptor neurons (ORNs) employ an odor receptor (OR)-coreceptor (Orco) receptor-ion channel complex for pheromone and general odor transduction, in the hawkmoth we find no evidence for ionotropic pheromone transduction. Instead, with in vitro and in vivo electrophysiological recordings of nocturnal hawkmoth ORNs we find evidence for a G-protein coupled pheromone-transduction cascade involving phospholipase C activation. Furthermore, we find daytime-dependent modulation of the transduction cascade controlled via endogenous clocks, such as circadian clocks in ORNs.

Since the molecular mechanism and members of insect pheromone/odor transduction and its circadian modulation are unclear, here, we investigated dynamic transcriptomic changes in adult male hawkmoth antennae at different zeitgeber times (ZTs) comparing sleep- and activity phases, in search for core hub genes of clock-regulated pheromone transduction. Preliminary studies identified an array of potential participants in pheromone transduction, encompassing 7 G-proteins, 123 ion channels, 43 participants of second messenger cascades, and 90 enzymes that potentially participate in pheromone transduction, which are implicated as candidate genes, and transcript variants. Furthermore, the circadian clock including period, timeless, clock, cycle, cryptochrome, and other 11 candidate genes, exhibited significant differences across different ZTs, suggesting circadian clock-dependent regulation of pheromone transduction. Subsequently, we will perform functional assays to challenge the role of these candidate genes in pheromone transduction, utilizing Ca²⁺ imaging, patch clamp, pharmacology, CRISPR/Cas, and RNA interference (RNAi), also in cockroach antennae.

In summary, this study elucidates the core hub genes involved in the clock-regulated pheromone transduction in *M. sexta*, contributing to our understanding of the molecular mechanisms and physiological regulatory network underlying insect olfactory perception.

Knockdown of the molecular circadian clockwork in the Madeira cockroach *Rhyparobia maderae* deletes behavioural circadian rhythms

Huleg Zolmon¹, Patrick Przybylla¹, Romy Freund¹, Thore Völker, Melissa Ballüer, Achim Werckenthin, Monika Stengl¹

¹University of Kassel

Organisms evolved endogenous clocks that allow for anticipation of environmental rhythms such as the daily light-dark cycle. Endogenous clocks are based upon positive feedforward and negative feedback loops orchestrating timing of rhythms in physiology and behaviour. The molecular clockwork of circadian clock neurons controlling sleep-wake cycles is studied best in the insect *Drosophila melanogaster*. It is based upon transcriptional-translational feedback loops (TTFLs) of clock genes. The transcription factors Clock (CLK), or Cycle (CYC) are positive feedforward elements activating transcription of the negative feedback elements Period (PER) and Timeless 1 (TIM1) that suppress their own transcription. Circadian TTFL clockworks of mammals and some insects such as the Madeira cockroach *Rhyparobia maderae* employ Cryptochrome 2 (CRY2) as another negative feedback element. In the Madeira cockroach transplantations identified the brain's accessory medulla (AME) as clock network controlling sleep-wake cycles. Its circadian neurons' clockwork is only partly known, with the negative feedback elements PER, TIM1, and CRY2. So far, RNA interference (RNAi) induced individual knockdown of all known negative feedback elements did not delete circadian sleep-wake rhythms. Furthermore, modelling studies suggested that there is an additional negative feedback element missing and that TTFL clockworks differ in distinct AME clock neurons. Since in mammals only deletion of CYC caused behavioural arrhythmicity, we first identified the positive feedforward elements CLK and CYC, before attempting to stop the TTFL clockwork with RNAi. Here, we identified the genes *clk* and *cyc* of the positive feedforward loop of the TTFL clockwork in the Madeira cockroach. Furthermore, via RNAi induced single and double knockdowns of their respective mRNAs we succeeded to disrupt circadian sleep-wake rhythms in *R. maderae*. Future genomic analysis and, also transcriptomic studies at the single cell level will reveal the structure and function of molecular clockworks in individual clock neurons of the AME clock.

The plasma membrane as multiscale posttranslational feedback loop oscillator

Jan Bröckel, Anna C. Schneider, Sohail H. Shoaib, Susanne Neupert, Monika Stengl, Friedrich W. Herberg

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The ability to predict environmental changes such as daily light-dark cycles increases the chances for organisms' survival. Thus, endogenous clocks, such as circadian clocks that anticipate the 24h light-dark cycles, evolved to translate the external environmental rhythms into an intracellular response. Two different types of clocks can be distinguished: transcriptional translational feedback loop (TTFL)- and post transcriptional feedback loop (PTFL)-clocks. They generate oscillatory outputs that couple a plethora of multiscale clocks throughout the body via unknown mechanisms.

In insect peripheral and central clock neurons we study components and mechanisms of plasma membrane associated multiscale oscillations, such as oscillations in membrane potential, intracellular Ca^{2+} , and cyclic nucleotide levels, searching for multiscale interactions. Both central peptidergic clock neurons of the Madeira cockroach and hawkmoth olfactory receptor neurons, which are peripheral circadian clocks, express circadian and ultradian membrane potential oscillations via unknown mechanisms. While patch clamp and extracellular recordings of primary cell cultures of neurons are established, and routine recordings of intracellular Ca^{2+} levels are performed with calcium indicator dyes such as Fura-2, we establish parallel FRET recordings with biosensors like Epac-SH187 and cGi-500 to measure cyclic nucleotide level changes in vivo and in real-time.

We predict that ultradian and circadian membrane potential oscillations are generated via pacemaker channels together with antagonistic channels constituting a PTFL clock. This membrane clock generates potential oscillations accompanied by intracellular Ca^{2+} oscillations via voltage-dependent Ca^{2+} channel activation. Cycling intracellular Ca^{2+} levels are hypothesized to drive cAMP production periodically via Ca^{2+} /calmodulin-dependent adenylyl cyclase, activating membrane-anchored protein kinase A. With various biochemical assays we examine spatiotemporal regulation and crosstalk of Ca^{2+} and cAMP signaling cascades on the circadian and ultradian scale in biological clocks and challenge our hypothesis of the plasma membrane as an endogenous PTFL clock that is coupled to, but not driven by, the TTFL nuclear clockwork.

Modeling and Simulations of intra-cellular Biochemical Mechanisms

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Spatio-temporal control of components of intra-cellular signaling cascades (e.g. biochemical reactions involving second messengers, enzymatic reactions, and ion currents) regulates a plethora of physiological processes and is thought to play a role in cell time-keeping mechanism. Mathematical modeling can help to understand how such signaling networks can generate oscillations and respond to incoming signals. The presented work contains two examples of modeling and simulation of intra-cellular signaling networks: first a data-based temporal model for the process of binding cyclic guanosine monophosphate (cGMP) to protein kinase G (PKG) is developed, which discovers detailed characteristics of the process; next an spatio-temporal model for oscillatory behavior of intra-cellular Calcium ion (Ca^{2+})-cyclic adenosine monophosphate (cAMP) pathway is presented, which is able to show how far a wave of the second messengers can travel in the cytoplasm. Mathematical modeling of signaling networks requires identification of the properties of the system based on experimental data. The unknown parameters of the model for the binding process (a system of linearized ordinary differential equations (ODEs)) is estimated, and the ODEs are solved analytically. The partial differential equation (PDE) is solved numerically using adaptive finite element method (FEM).

A stochastic conductance-based model of the hawkmoth *Manduca sexta* olfactory receptor neuron

Mauro Ariel Forlino, Aditi Vijayan, Katrin Schröder, Anna C. Schneider, Monika Stengl, Martín García

University of Kassel

The long trichoid sensillum in male hawkmoths, *Manduca sexta*, is innervated by two olfactory receptor neurons (ORNs) that respond to the pheromone released by female moths to attract conspecific mates. In the absence of odor stimuli, pheromone-sensitive ORNs in hawkmoths exhibit non-randomly distributed spontaneous spikes. These spikes occur within bursts and between bursts, and it is our goal to understand the interactions between the ion channels responsible for their generation. Therefore, analyzing spike distribution within and between bursts, as well as burst frequency and duration, is crucial for identifying different mechanisms at play. The random opening and closing of ion channels introduce internal fluctuations in neurons, known as channel noise, which contributes to the variability in spike distribution and determines whether a single spike or a burst occurs. Furthermore, insect ORNs serve as endogenous peripheral circadian clock neurons, leading to the expression of daytime-dependent rhythmic spike distributions.

In this study, we present a novel conductance-based model that incorporates the olfactory receptor coreceptor (ORCO) as a pacemaker ion channel with linear conductance dependent on cAMP concentration. Our model takes into account that cAMP express daytime-dependent rhythms with concentration being maximal during activity phase. By utilizing stochastic differential equations based on the microscopic Markovian states of ion channels, our model can reproduce the observed spike distribution with its circadian oscillations.

Machine Learning Conservation Laws of Dynamical systems

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Conservation laws are of great theoretical and practical interest. We describe a novel approach to machine learning conservation laws of finite-dimensional dynamical systems using trajectory data. It is the first such approach based on kernel methods instead of neural networks which leads to lower computational costs and requires a lower amount of training data. We propose the use of an "indeterminate" form of kernel ridge regression where the labels still have to be found by additional conditions. A simple approach minimises the length of the coefficient vector to discover a single conservation law. A more sophisticated approach uses gradients to construct step by step a complete set of functionally independent conservation laws

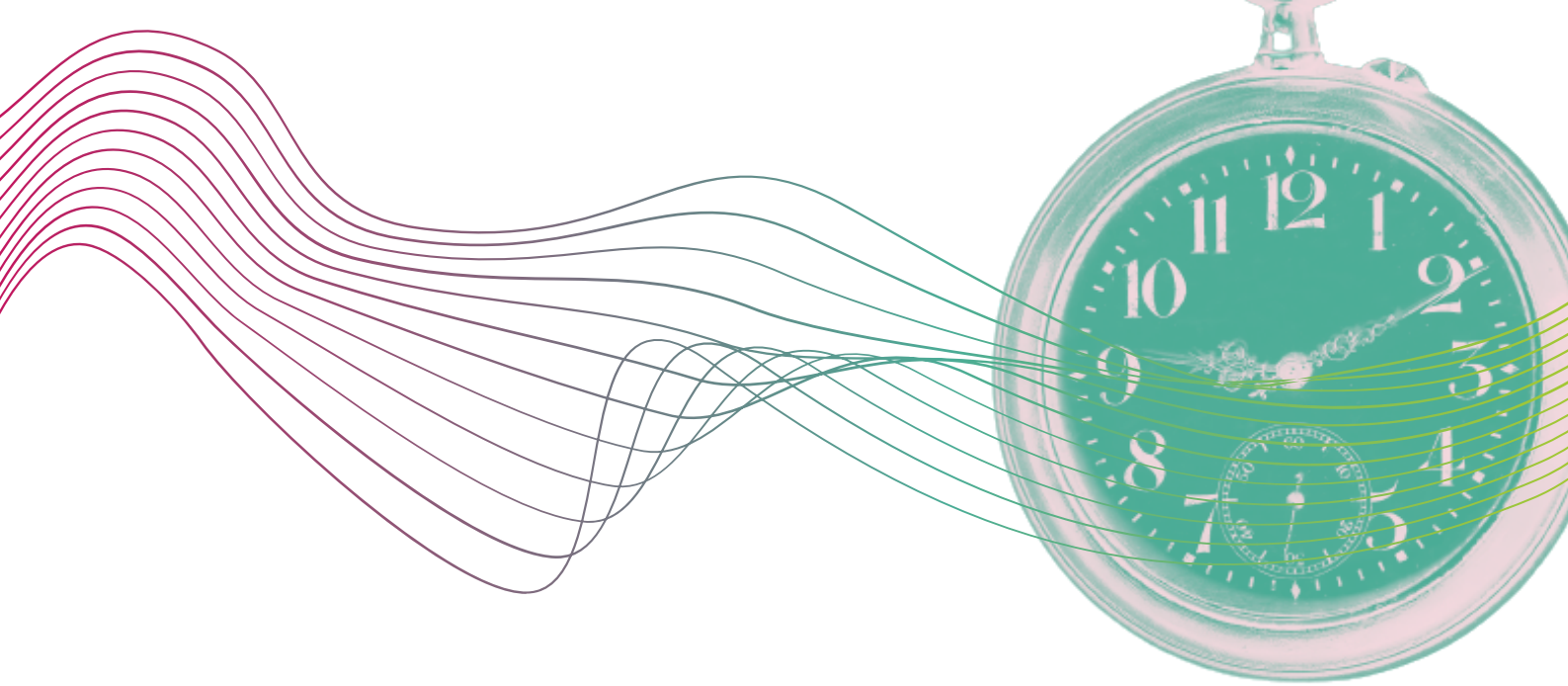
Light entrainment pathways to the circadian clock circuit of the Madeira cockroach *Rhyparobia maderae* controlling sleep-wake cycles

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Endogenous circadian clocks evolved in organisms orchestrating rhythms in physiology and behavior, entrained to the daily light dark cycle. For the night-active Madeira cockroach *Rhyparobia maderae*, transplantation studies located the circadian clock that controls sleep-wake cycles to the accessory medulla (AME) in the brain's optic lobes. The neuronal circuit of the AME in each of the bilaterally symmetric optic lobes of the cockroach brain comprises two negatively coupled oscillator circuits. The morning (M) oscillator is advanced by light pulses at dawn, while the evening (E) oscillator is delayed by light pulses at dusk. All four oscillators of the brain couple with each other to orchestrate together the daily sleep wake cycle. Since the night active cockroach sleeps during the day, the M oscillator appears to be sleep promoting, while the E oscillator is activity promoting.

Neither in the mammalian nor in the insect's circadian clocks it is understood which neuronal mechanisms entrain the neuronal circadian clock network to the daily light dark cycle and how light input into the clock is gated. Since behavioral experiments suggested that UV light is sleep promoting and green light activity promoting, we hypothesized that UV light advances the M and green delays the E clock circuits. To challenge our hypothesis of parallel light entrainment pathways to different clock circuits, behavioral assays were performed in different light regimes. Furthermore, we developed a simplified clock network model based on switched differential equations to describe the oscillatory behavior of four coupled oscillator circuits. Preliminary behavioral experiments showed a tendency of period lengthening in constant green and period shortening in constant UV light, before periods changes slowly reversed. With further behavioral assays combined with model-based analysis, we attempt to refine our hypothesis of interconnections between parallel light entrainment pathways to the M and E clock circuits.



SUBJECT GROUP INVITED TALKS



ManyPrimates - An infrastructure for collaboration in primate cognition research

Manuel Bohn

Max Planck Institute for Evolutionary Anthropology

Human cognition has a long evolutionary history, most of which is shared with our primate relatives. By comparing different primate species, we can study the evolutionary dynamics that lead to the emergence of cognitive abilities. However, inferring the evolutionary history of cognitive abilities requires large and diverse samples, both in terms of species as well as individuals. Yet, such samples are often beyond the reach of individual researchers or institutions, and studies are often limited to small numbers of species. Here we present the ManyPrimates project, which addresses these challenges by providing a large-scale collaborative framework for comparative studies in primate cognition. We demonstrate the viability of this approach in a first study on short-term memory in primates. We were able to include 400+ individuals from 40+ primate species housed at 29 sites across Africa, Asia, North America and Europe. All subjects were tested in a delayed-response task using consistent methodology across sites. Our analyses demonstrate a considerable contribution of phylogeny over ecological and social factors on the distribution of short-term memory performance in primates, closely related species had more similar short-term memory abilities. Our results demonstrate the feasibility of a large, collaborative open-science project. Since then, we have worked on three additional projects on diverse topics such as delay of gratification, inference by exclusion and tool use. With this talk, we hope to further spread the word about the project.

Phylogeny-ontogeny relations in the cell type evolution across Metazoa

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A cell type represents one of the fundamental units of evolution, and the diversity of cell types underlies the diversity of animal species. Yet, how individual cell types evolve remains poorly understood. We present a novel approach that integrates genomic phylostratigraphy with single-cell transcriptomics to understand the individuation of cell type-specific molecular programs across the tree of life and along ontogeny.

Using scRNAseq data from the cnidarian *Hydra* and the zebrafish *Danio rerio*, we provide evidence that on the transcriptome level, the phylogenetic history of a cell type is robustly reflected in its ontogeny: across animal species, differentiated cells of diverse origin express a progressively younger transcriptome. Importantly, this pattern is characteristic not only for lineage-restricted cell types, such as Cnidaria-specific nematocytes but also for evolutionary ancient cell types, such as epithelial cells and neurons. We demonstrate that the phylogeny-ontogeny correlations previously described on the organismal level, known as the developmental hourglass model, are not an emergent property of the animal embryos. Instead, these patterns reflect the sequential activation of gene-regulatory programs along the differentiation of each cell lineage.

Our approach allows uncovering systematically and across large phylogenetic distances the assembly of cell types' molecular programs. It reveals the contribution of specific ancient functional modules as well as novel, lineage-restricted genes. Importantly, such unbiased tracing of cell types' evolutionary history generates predictions on the functional relevance of specific genes and their ensembles, which can be experimentally validated. This approach may help resolve long-standing questions of cell type evolution and provide insights into major evolutionary innovations that drive Metazoa radiation.

Behavioral evolution in a complex world of flies, food, and love

Philipp Brand

Rockefeller University

From dancing birds to singing flies, animals have evolved an astounding diversity of behaviors to attract and choose mating partners in their natural habitats. Guided by innate preferences and aversions, the brain filters complex environments for cues and signals to successfully navigate these reproductive behaviors in appropriate spatial, temporal, and social contexts. How does the environment shape sensory evolution and contribute to the diversification of mating behaviors? Using the highly tractable nervous system of *Drosophila* and other insects as an inroad, I study the neural mechanisms underlying the evolutionary ecology of reproductive behaviors and how the environment contributes to their rich diversification.

In *Drosophila*, courtship and mating occurs on fermenting food where many individuals congregate. Replicating this naturalistic context in the lab, I discovered striking differences in the sensory dependence of mating behaviors across species. Most notably, *Drosophila erecta* requires the presence of food to engage in courtship and only mates in social groups. Using a combination of experimental ethology, in vivo functional brain imaging, genetic manipulation, and optogenetics across species, I revealed that sexual arousal in *D. erecta* is uniquely gated by food odors that switch the valence of visual stimuli leading to courtship and mating. This provides a possible neural mechanism underlying the environmental modulation of reproductive behaviors, highlighting how social behaviors are shaped by the natural environments in which they evolve.



Are long lived parasites more virulent?

Charlotte Rafaluk-Mohr

Freie Universität Berlin

Why do parasites and pathogens harm their hosts? This is a question that has been of interest to evolutionary biologists for decades. The most widely accepted answer to this question comes from the trade-off hypothesis, which posits that parasites must cause harm in order to replicate and extract resources from their hosts. Too rapid reproduction and the host could die before transmission to a new host is possible, too slow reproduction would mean a loss of exploitation opportunity and thus fitness and thus an intermediate level of virulence is expected to evolve. In spite of this, we see examples of very high virulence in nature that appear to be evolutionary stable. One potential explanation for this is that certain groups of pathogens are able to circumvent the costs of high virulence. Pathogens who are able to survive for long periods of time in the environment, for example, may avoid some of the costs of high virulence as, even if they kill their current host, they can wait in the environment for new susceptible hosts to migrate. Evidence regarding this relationship, is however mixed, with some results supporting an alternative hypothesis, that survival in the environment is costly and thus traded-off against virulence. We have been attempting to resolve this controversy using evolution experiments carried out in two separate invertebrate host – fungal pathogen host-pathogen model systems. Initial results on a panel of *Metarhizium* isolates infecting *Tribolium castaneum* beetles show support for a positive relationship between environmental persistence and virulence, however, selection experiments in the two systems show that, while there does seem to be a link between the two traits, which under certain circumstances results in a positive evolutionary relationship, the picture is more complicated and other pathogen life-history trade-offs, beyond those between virulence and transmission appear to impact optimal virulence.



The enigmatic world of mud dragons (Kinorhyncha): insights into morphology, development, and evolution

María Herranz

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Kinorhyncha, commonly known as mud dragons, is an enigmatic phylum of marine, microscopic organisms that thrive in diverse habitats worldwide ranging from intertidal to hadal depths. Kinorhynchs are part of the 'molting animals' known as Ecdysozoa and are closely related to loriciferans and priapulids forming the Scalidophora clade. The body plan of Kinorhynchs consists of a retractable head with radial symmetry, a neck, and a limbless cylindrical trunk composed of eleven articulated segments.

There are multiple aspects of mud dragons that remain largely unknown, particularly in their reproduction and development. Kinorhynchs have separate sexes with internal fecundation, females lay one egg at a time that is hidden in the sediment. The development is direct, lacking larval stages and hatching from eggs almost as miniature versions of adults. The first juvenile stage (J1) hatches with a well-developed head, a neck, and at least eight out of the eleven final trunk segments. It then progresses through five additional stages (J2-J6) before reaching adulthood. During post-embryonic development the trunk segments are sequentially added in a subcaudal region with the terminal segment initially elongating and then undergoing transverse division. However, the molecular mechanisms underlying these basic developmental processes are completely unknown. Over the last decade my research has focused on pushing the boundaries to understand important aspects of kinorhynch biology, employing state of the art techniques. Here I present new insights into kinorhynch morphology, reproduction and post-embryonic development based on live observations, DIC-LM, SEM and CLSM data. All combined with cell proliferation and differential gene expression analyses.

Social modulation of threat-evoked defensive responses

Clara Ferreira

Northumbria University

A major benefit of being in a group is the possibility of adding social information to directly-perceived information about the environment to guide behaviour. Across the animal kingdom, social information acquired via specific signals or cues animals produce as they engage in their daily activities are used for decisions in reproduction, foraging and protection against predation. Acute fitness benefits of the usage of this information are flagrant in the context of a response to a potential threat: failure to detect a predator can lead to an animal's immediate demise whereas needless engagement in metabolically costly defence responses can, unnecessarily, negatively impact survival. We previously showed that *Drosophila melanogaster* can use social information to modulate their active immobility responses to an external visual threat, i.e., freezing, aimed at becoming inconspicuous. These freezing responses are upregulated when surrounding others freeze or downregulated when surrounding others move. With tight control over the surrounding social environment by genetically controlling the movement of others, we are now exploring the interplay between these social cues of danger and safety and uncovering the mechanisms by which they modulate the freezing response deploying circuitry.

How to respond to low oxygen: O₂-sensing in mammalian cells and tissues

Carsten Scholz

Universitätsmedizin Greifswald

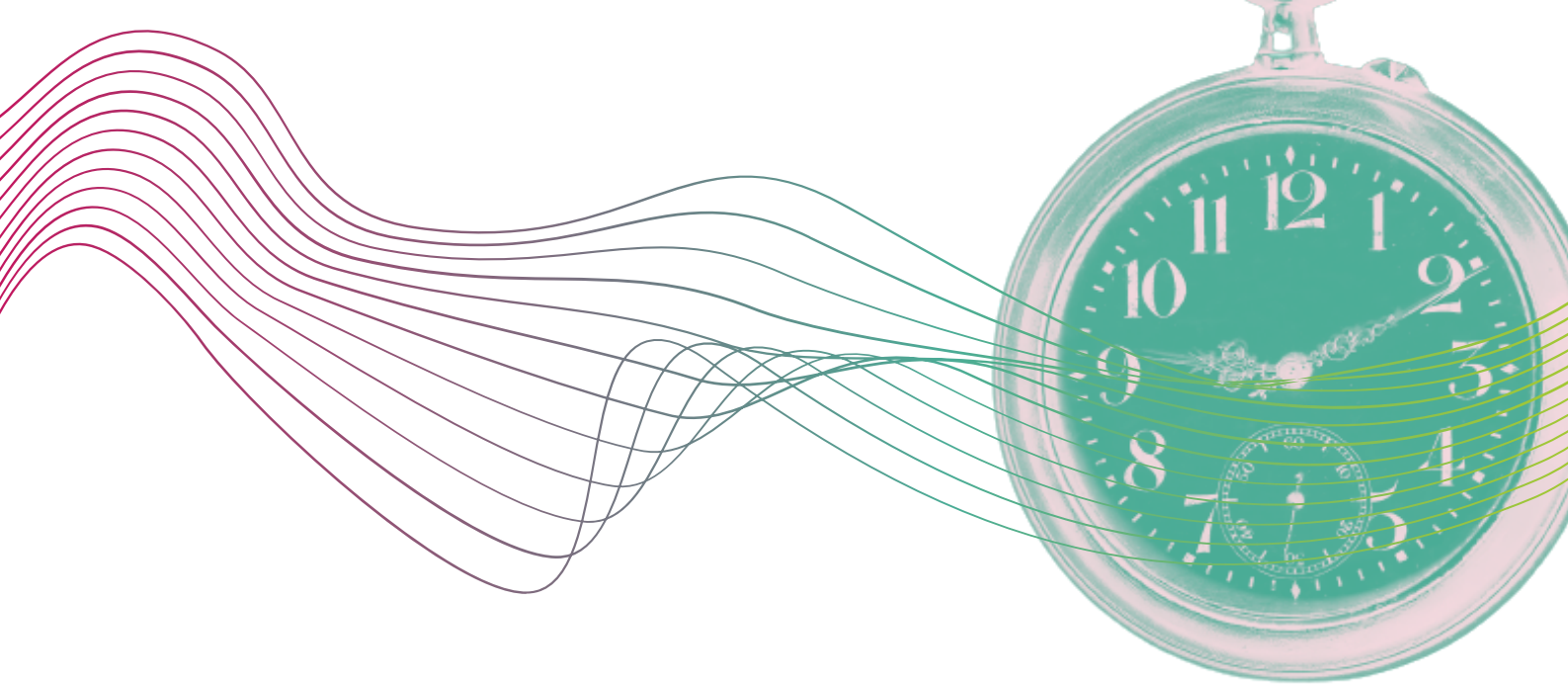
Oxygen depletion (hypoxia) is a frequently encountered condition in both physiology and disease. Metazoans have evolved several cellular mechanisms to sense decreases in local oxygen availability and to mount adaptive responses to survive. The best characterised cellular oxygen sensing occurs via protein-4-hydroxylases 1-3 (PHD1-3) and factor inhibiting HIF (FIH). These four enzymes hydroxylate α subunits of the dimeric transcription factor hypoxia-inducible factor (HIF) in an oxygen-dependent manner. In normoxia, these hydroxylations lead to the degradation of HIF- α as well as to the inhibition of its transactivation activity. In hypoxia, HIF- α is no longer hydroxylated, escapes its degradation and inactivation, and enhances the transcription of up to 1,000 genes. One of the most prominent HIF target genes is erythropoietin (Epo), which regulates the production of erythrocytes and thus the oxygen carrying capacity of the blood. Recently, pharmacologic inhibitors of the HIF hydroxylases have entered the clinics for the treatment of renal anemia due to their capability to induce Epo transcription. In the last years, additional cellular oxygen sensors and oxygen sensing mechanisms have been proposed. We recently suggested that the HIF hydroxylase FIH can form oxygen-dependent stable protein oligomers (oxomers) with substrate proteins, regulating cellular energy metabolism. In addition, histone lysine demethylases (KDM) have been suggested as novel oxygen sensors that regulate transcription by removing lysine methylations. Another possible cellular oxygen sensor is the enzyme cysteamine (2-aminoethanethiol) dioxygenase (ADO), affecting the N-degron pathway in an oxygen-dependent manner. Thus, our knowledge about how cells sense and respond to hypoxia is greatly expanding, increasing our understanding of oxygen physiology in metazoans and opening up avenues for the development of novel therapeutics for hypoxia-associated diseases in humans.

The reconstruction of anthropogenic change

Christine Ewers

Christian-Albrechts-Universität zu Kiel

Human actions have changed the global ecosystem irrevocably. To understand the consequences of these changes, we need to reconstruct the past. This baseline allows us to evaluate which species are changing in abundance or distribution, genetically or ecologically. Reconstructing the past is an inherently difficult endeavour, as we cannot actually go back in time and sample past populations. However, natural history museums contain many “Zeitzeugen” that we can interrogate with novel molecular, morphological and statistical approaches. I will talk about my work on historical collections to reconstruct species extinctions and anthropogenically driven range expansions in molluscs and crustaceans.



SUBJECT GROUP TALKS



Reconstructing the visual capacity of the miniature silverleaf whitefly, *Bemisia tabaci*

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In the course of evolution, many insect species have gone through a process of miniaturization resulting in body lengths of under a millimeter. This phenomenon leads to the simplification and reduction of systems in the insect's body such as the compound eye, which underwent a structural reduction in the number and/or size of ommatidia. As the optical resolution of compound eyes depends on the number of ommatidia and their physical properties, small animals should be limited in their visual capacity for object recognition and optomotor behaviors. Here we present a detailed evaluation of the unique two-part eye structure of the millimeter-long silverleaf whitefly, *Bemisia tabaci*. Whitefly heads were three-dimensionally reconstructed from μ CT scans and the eyes were analysed using self-written MATLAB code. From the digitized coordinates of morphological markers, we derived the geometrical properties of the eye and ommatidia including interommatidial angles and visual axes (gaze). We further determined the position of the eye during locomotion by analyzing high-speed videos of walking, free-flying, and tethered-flying whiteflies. These analyses allowed us to virtually construct ommatidial projection maps according to eye position. We found that the 100 μ m long, two-part compound eyes consist of approximately 70 ommatidia and that visual resolution and ommatidia properties differ between the upper and bottom parts of the eye. Furthermore, we revealed that eye orientation is determined by body angle as the head does not move independently during flight. As the eyes changed orientation during locomotion the bottom part dramatically changed its area of focus while the upper part focused constantly on the horizon. This, together with the geometrical differences between the two parts, implies a division in functionality between the two parts. Altogether, our study uncovered several novel properties of insect miniature eyes and predicts a reduction in control capacity during vision-guided flight behaviors.

Drosophila species from high latitudes can adapt their daily activity well to long photoperiods, but this ability does not depend exclusively on the neuropeptide F

Peter Deppisch; Francesca Sara Colizzi; Charlotte Förster

Julius-Maximilians-Universität Würzburg

Light is the most potent Zeitgeber for the *Drosophila* circadian clock. At high latitudes it plays an even more important role as flies must measure daylength to prepare for changing seasons and adapt their physiology accordingly. Daylength measuring can be achieved by evaluating the phase difference between two oscillators that track dawn and dusk – also called morning (M) and evening (E) oscillators. In the fruit fly *Drosophila melanogaster*, such M and E oscillators exist and are well characterized on neuronal and behavioral levels. We recorded the locomotor activity rhythms of 52 different species of the genera *Scaptodrosophila* and *Drosophila* from different geographical locations in long photoperiods, checked for the presence of M and E activity peaks and investigated their ability to track dawn and dusk. We found that most species showed M and E activity and that species that have spread to more northern regions tracked dawn and dusk better than species that are restricted to tropical zones. We then investigated the expression pattern of the neuropeptide PIGMENT DISPERSING FACTOR (F), which seems important for delaying E activity. Despite the tendency of most high-latitude species to have more F positive terminals in close vicinity to putative E oscillator neurons, we could not see a considerable correlation between F expression patterns and the geographical origin. F was also found to be important for robust rhythmicity. Several northern species lack F in the small ventrolateral neurons and possess consequently weak circadian clocks. Weak clocks facilitate clock phase shifting, enabling E activity to track dusk. Nevertheless, not all high-latitude species with E activity tracking dusk possess weak clocks and lack F in these neurons. We conclude that flies have evolved different ways to adapt to high latitudes, which do not generally involve F.

A circumplex model of affect in *Drosophila*

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University of Würzburg, Biocenter, Theodor-Boveri-Institute

The open field test (OFT) is widely used to score anxiety and depression levels in rodents. In the OFT, fruit flies show wall-following behavior (WAFO) similar to rodents. However, it is questionable to which extent insects are able to perceive emotions such as fear, anxiety or depression. Therefore, we set out to characterise the influence of emotion-like states on the OFT behaviour in flies. We measured both WAFO and total walking (TOWA) in the OFT in wildtype flies paired with negative or positive stimuli, altered internal states or pharmaceutical treatment.

CantonS wildtype flies treated with negatively valenced biological stressors including social isolation, starvation, sex deprivation, and sleep deprivation showed an increase in WAFO and TOWA. This increase was reversed after removal of the stressor. Similarly, WAFO and TOWA increased in CantonS flies after aversive physical treatments including heat, mechanical shake and electric foot shock (EFS). Interestingly, after consecutive EFS (CEFS), only WAFO but not TOWA increased. Positive pharmacological treatment with 5mM diazepam decreased both WAFO and TOWA. Similarly, overexpression of the serotonin transporter and activation of reward neurons (dopaminergic PAM neurons, NPF neurons) decreased WAFO. In line, inhibition of PAM neurons increased WAFO. We further found a positive correlation between WAFO and TOWA in various lab- and natural wildtype strains. A comparison between the activity in the OFT and *Drosophila* activity monitors (DAM) data suggests that TOWA is not correlated with the spontaneous activity level. In summary, our data suggests that OFT analysis reports emotion-primitives in flies which can be surprisingly well described by the circumplex model of affect. This widely accepted model in psychology classifies human emotions by two bipolars (valence and arousal). The results now allow us to study the role of neuromodulators and intracellular signaling pathways on emotion-like states in *Drosophila* using neutral terminology.



Picking up good vibes: Variation of vibratory signals and the evaluation of signal quality in the spider *Pisaura mirabilis*

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Substrate-borne vibration is a channel of communication which is often overlooked yet remarkably common in the majority of arthropods, and especially in arachnids. The male Nursery Web Spider, *Pisaura mirabilis*, uses vibratory signals (in addition to chemical and visual stimuli) in mate attraction and courtship. These courtship vibrations consist of repeated pulses, and given their notable energetic cost, provide an honest advertisement of the male's physical condition. Our project explores the functional role of vibratory communication within the framework of *P. mirabilis* reproduction. First, we did a deep-dive into the male vibratory courtship signal, evaluating individual variation in signaling behavior based on repeated measurements (N = 3) of 150 spiders. We found that our population of male spiders showed notable between-individual variation, as well as high levels of within-individual consistency. By utilizing measurements of pulse rate and pulse interval consistency, two temporal variables which putatively affect female choice, we were able to evaluate the quality of the males' vibrational output as "high-" or "low-signaling." We applied our screening method to categorize a new population of males (N = 120), selecting only the highest- and lowest-ranked quartiles for use in mating experiments. Female spiders (N = 60) were then mated twice in sequence- once with a high-signaling and once with a low-signaling male. Fitness effects (number of live, dead, and unfertilized offspring) were gathered from these mating trials, and future molecular testing will elucidate whether the quality of males' vibratory signals has an effect on their mating success.



Talking to the kids - parental signalling in the burying beetle *Nicrophorus vespilloides*

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¹ University of Bayreuth; ² CBC (Europe) S.r.l.

Communication plays a vital role in any form of cooperative behaviour for numerous interactions. Apart from odour, acoustic signalling is common during various behaviours such as mating, defence, aggregation, and parental care - a prime example for cooperative behaviour. One group that exhibits elaborate biparental care are burying beetles of the genus *Nicrophorus*, which makes them model organisms in behavioural ecology. Although their stridulations have been known since Darwin, and both parents stridulate, their actual function remains unclear to this day. In this study, we investigated the function of their stridulatory signals during brood care in *N. vespilloides*. To examine the role of communication during brood care, we recorded biparental and uniparental parents over the entire brood care and observed their stridulation activity as well as various signal parameters. We found that stridulation activity significantly increased after larval hatching and that there is no difference between uniparental and biparental couples in stridulation activity meaning that after hatching most communication is directed towards the offspring. We could also show that signal parameters after hatching showed a much higher variance in peak frequency pointing towards different signals being produced. This is evidence for intricate parent-offspring communication which we know from previous studies to be essential for offspring survival and weight gain. Such complex parent-offspring communication is mainly known from vertebrates so far and these findings shed a new light on the importance of communication during parental care.

Impact of inbreeding and parental care on offspring performance in the biparental cichlid fish *Pelvicachromis pulcher*

Timo Thünken

University of Bonn

Inbreeding, i.e. mating between relatives can lead to inbreeding depression. While it is well known that inbreeding-effects can be environment-dependent and that harsh environmental conditions can increase negative inbreeding effects, the capacity of the social environment to buffer inbreeding depression is less well studied. Here, we examined the (long-term) effects of parental care on juvenile performance of inbred and outbred offspring at different life stages in the biparental cichlid fish *Pelvicachromis pulcher*. A previous study revealed that this species does not avoid but tolerate inbreeding. We conducted a series of experiments, in which we compared the survival and the social behaviour of inbred and outbred offspring that were reared with or without parents. Our results suggest inbreeding depression with respect to survival at early life stage, which however, could be buffered by parental care. Inbreeding did not affect survival or social behaviour of older, independent juveniles. In contrast, our results indicate persistent parental effects on juvenile aggression, shoaling behaviour and shoal choice. In conclusion, our studies suggest that inbreeding and the social environment (interactively) affect survival, social behaviour and potentially the cognitive abilities of the offspring.

Adaptive predator-avoidance behavior in a globally introduced bird species

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Studying invasive species provides valuable insights into the processes of ecological adaptation. These species face both challenges and opportunities in novel environments, which shape their morphological and behavioral traits that facilitate successful invasion. One important adaptive behavior is predator-avoidance, which allows species to adjust to local novel conditions. In this study, we investigated predator-avoidance behavior in the Common Myna (*Acridotheres tristis*), a globally successful avian invader, using the Flight Initiation Distance (FID) assay where a human observer acts as a potential predator. We examined the FID behavioral responses of the Common Myna across its invasive range and analyzed predictors of escape behavior. Our findings revealed that FID increased with greater starting distance, lower immediate human density, flighted escape responses compared to walking responses, and at lower perching heights above ground. Interestingly, FID was not influenced by myna group size. Functionally, we observed that FID increased in areas located more towards the rural end of an urban-rural gradient, at shorter distances from the Equator, and with greater time since introduction. Furthermore, we conducted a detailed examination of FID responses in a recent invasion (~25 years) in Israel, comparing invasive mynas with local house sparrows (*Passer domesticus*). We found that invasive mynas exhibited shorter escape distances compared to native sparrows, especially in areas with higher human density. These results provide insights into the sensory cues that trigger anti-predator behavior in invasive mynas and highlight the adaptive patterns of anti-predator responses in a globally introduced species. Overall, our findings demonstrate the remarkable behavioral flexibility of the Common Myna, which allows it to maximize foraging efficiency in variable environmental conditions and contributes to its successful global invasiveness. This study sheds light on the adaptive mechanisms underlying invasive species' ecological success and highlights the importance of understanding predator-avoidance behaviors in the context of biological invasions.

What is shadow competition and where can it be found?

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Competition is a fundamental process in ecology and evolution. Shadow competition is a competition type, where a predator intercepts an approaching prey item earlier in its trajectory, preventing it from reaching the attack range of predators downstream. Although shadow competition is probably common in nature, there are only sporadic reports of it in the scientific literature. The necessary involvement of space and movement direction differentiates shadow competition from other classical competition types, like interference and exploitation. We will survey cases of shadow competition in nature and emphasize also cases in which it should be present but is unreported. We will explain its contribution to the understanding of predator-prey dynamics. Next, we will discuss mechanisms that can increase or moderate the strength of shadow competition and will describe an examination of four such mechanisms using an individual-based spatially-explicit simulation model. One simulated scenario is when prey capture is uncertain. The strength of shadow competition increases when it is harder to capture prey after the first unsuccessful attempt. Shadow competition is also stronger when predators can capture prey arriving from only one direction. In three-level systems of plants in clusters, herbivores searching for plants, and predators ambushing for herbivores inside plant clusters, ambush locations of predators in the periphery of plant clusters are more successful than those at the cluster centre, especially as predator density goes up. In conclusion, competition is a fundamental process in ecology, and shadow competition is a crucial but understudied aspect of it. Understanding the factors that affect shadow competition and its impact on predator-prey dynamics could bring significant contributions to the field of ecology and animal behaviour.



A population of feeding-responsive, multipotent stem-like cells in the sea anemone *Nematostella*

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Most animals exhibit life-long, indeterminate growth that is mainly dependent on nutrient availability. In animals with fixed body sizes, which includes most genetic research organisms, body growth arrests at maturity and becomes uncoupled from food availability. Therefore, it is currently only poorly understood how feeding regulates animal growth. Here, we used the sea anemone *Nematostella vectensis* to study the cells and molecules underlying its nutritional growth regulation. We found that feeding fuels exponential growth and induces a burst of body-wide cell proliferation. In contrast, starvation led to a profound cell cycle arrest and body shrinkage, which could both be quickly reversed by re-feeding. Aiming to study the cellular basis of feeding-controlled growth plasticity, we found a multipotent, Vasa2+/Piwi1+ stem-like cell population in *Nematostella*. First, we studied their fate by generating transgenic vasa2 and piwi1 reporter lines using random integration and CRISPR/Cas9-mediated genomic knock-in technologies. Strikingly, we found that Vasa2+/Piwi1+ cells generate both the germline and a diversity of somatic cells, including soxB(2)+ neuronal progenitors, throughout the juvenile and adult polyp. In addition, we found that the proliferation of both Vasa2+/Piwi1+ stem-like cells and their progeny are strongly regulated by feeding and starvation. Our results suggest that juvenile Vasa2+/Piwi1+ cells represent a multipotent stem-like cell population that underlies feeding-dependent body plasticity in *Nematostella*.



Developmental repatterning of the nasal-chemosensory organs during the evolution of terrestrial reproductive modes in frogs

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What is the role of developmental repatterning during the origin of evolutionary novelties? An excellent model system to approach this question is the evolution of diverse reproductive modes in anurans. In anurans, the ancestral reproductive mode involves aquatic eggs and free-swimming feeding tadpoles that develop via a thyroid hormone dependent metamorphosis into more-or-less terrestrial adults. This transformation results in the extensive remodelling of aquatically adapted organs to function within the terrestrial environment. This reproductive mode has been altered independently in many anurans leading to different degrees of terrestrial reproduction that include terrestrial eggs and non-feeding terrestrial tadpoles as well as direct development and viviparity. Here we study the development of the nasal-chemosensory system in anurans with different reproductive modes. The nasal-chemosensory system consists of the olfactory and the vomeronasal organ. In frogs showing the ancestral reproductive mode, the nasal-chemosensory system of the tadpole is specialized to function in an aquatic habitat. The system is extremely remodeled during metamorphosis, including the development of new chambers, ducts and gland types, enabling functionality in a terrestrial habitat. How does this developmental pattern change during the evolution of increasingly terrestrial reproductive modes (terrestrial biphasic and direct development) and viviparity? To answer this question we used histology and μ CT-based 3D-reconstruction of the developing nasal-chemosensory system in frogs with different reproductive modes. Our results show different developmental trajectories that result in a similar adult morphology. Furthermore, we investigated thyroid gland maturation as potential regulator of the timing of remodeling from the embryonic/larval into the adult nasal-chemosensory system.



Sex-specific differential gene expression focusing on reproduction during stolonization in the branching syllid *Ramisyllis kingghidorahi* (Annelida, Syllidae)

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Gene expression in the anterior end of syllid annelids has long been suggested to play key roles in sexual maturation. Thus, it is relevant to identify molecules involved in this process and address whether existing annelid models of reproduction can be generalized to Syllidae. Notably, despite hosting significant diversity in terms of reproductive strategies, gene regulation regarding the process of stolonization in the “Ribbon Clade” (Syllinae: Syllidae) is not well understood. Within the Ribbon Clade, *Ramisyllis* species markedly differ from all other Syllidae by their ramified bodies, their symbiotic lifestyle within sponges, and their stolonization mode. Here, we generated the first transcriptome assembly of *Ramisyllis kingghidorahi* (first for the Ribbon Clade) and performed comparative transcriptomics during stolonization to identify reproduction-related transcripts in specific sexes as well as body parts. Gene expression in the unbranched prostomium and proventricle (anterior ends), branched midbody, and stolons of male and female specimens was compared. The body region prostomium and proventricle showed not only the highest number of differentially expressed transcripts related to sexual processes, but also the most distinct set of transcripts when compared to other body regions. Genes related to reproduction, hormonal activity, and sexual maturation were particularly highly up-regulated compared to other body regions. In stolons, eye-related genes were highly expressed and gene expression differed significantly between sexes and body parts. While analyzing gene expression patterns between samples, different body regions accounted for greater variety than different sexes. In agreement with previous evidence, we argue that prostomium, proventricle and stolons all play distinct roles during reproduction.



Single cells or single nuclei? Efficient analysis of cell type diversity for low amounts of input material?

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Recent single-cell RNA sequencing (scRNAseq) approaches allow studying gene expression for thousands of individual cells on a genome-wide scale. Such approaches already provided valuable insights into the cell type composition of heterogeneous tissue samples of animal model systems and emerging models alike. A major prerequisite for a successful application of the method is the dissociation of complex tissues into individual cells, which often requires large amounts of input material and harsh mechanical, chemical and temperature conditions. However, the availability of tissue material may be limited for small animals, specific organs, certain developmental stages or if samples need to be acquired from collected specimens. Therefore, we evaluated different dissociation protocols to obtain single cells from small tissue samples of *Drosophila melanogaster* eye-antennal imaginal discs.

We show that a combination of mechanical and chemical dissociation resulted in sufficient high-quality cells. As an alternative, we show that the isolation of single nuclei is highly efficient for fresh and frozen tissue samples. We performed scRNAseq and single-nuclei RNA sequencing (snRNAseq) to show that the best protocols for both methods successfully identified relevant cell types. At the same time, snRNAseq resulted in less artificial gene expression that is caused by rather harsh dissociation conditions needed to obtain single cells for scRNAseq. A direct comparison of scRNAseq and snRNAseq data revealed that both datasets share biologically relevant genes among the most variable genes, and we show differences in the relative contribution of the two approaches to identified cell types.

In summary, if tissue availability is limited, we recommend the snRNAseq procedure of fresh or frozen tissue samples as it is perfectly suited to obtain thorough insights into cellular diversity of complex tissue.

Everything upside down in Oz? Flexible employment of torpor in squirrel gliders (*Petaurus norfolcensis*) as an adaptation to unpredictable climate

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Small mammals employ torpor for many reasons beside the obvious low temperature and lack of food during winter, e.g., to counter heat stress. The aim of our study was to investigate how free-ranging Australian squirrel gliders (*Petaurus norfolcensis*) are coping with seasonal and unpredictable environmental change, as there are no data on how small, potentially heterothermic mammalian species respond to a climate that experiences extreme conditions at both ends of the temperature continuum: heat waves in summer and cold spells in winter. We analysed how *P. norfolcensis* adjust their long- and short-term energetic budgeting to the profoundly varying conditions throughout the year by comparing possible seasonal heterothermic responses of the same species in the same habitat as adaptation to cold (to save on costs of endothermy) and hot conditions (to avoid overheating). Contrary to our expectations, squirrel gliders did not become torpid during winter or summer, but during spring. This is probably due to reproductive activities, which were more distributed throughout the year than is known from more consistent climates along the coast. Furthermore, amplitudes of euthermic daily fluctuations of body temperature showed great variability. Our results give insight into the physiological toolbox small mammals have at their disposal to cope with challenging conditions and will enable predictions about their capacity to endure environmental and climatic change.

Alcohol use by hornets

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Ethanol, a natural byproduct of sugar fermentation, is toxic to animals despite its high caloric value. However, certain animals, like tree shrews and fruit flies, consume low ethanol without negative effects up to a certain threshold of about 4%. We suggested that the mutualistic relationship between ethanol-producing organisms and social wasps might impact their ability to utilize ethanol. We fed Oriental hornets a sucrose solution with ethanol as their sole energy source and found that even at concentrations as high as 80%, ethanol consumption had no significant effect on hornet survival, behavior, or lifespan. Hornets readily consumed ethanol and did not exhibit a preference for sucrose over ethanol. Using $^{13}\text{C}_1$ labeled ethanol, we investigated the metabolism of ethanol in hornets compared to honey bees, revealing that hornets metabolize ethanol significantly faster. These findings suggest that hornets have a unique adaptation to ethanol consumption, potentially linked to their co-evolutionary history with ethanol-producing organisms.

InsectMow - Development and Evaluation of insect- and spider-friendly mowing technique

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Several studies show that a large proportion of spiders and insects living on agricultural grassland are damaged and/or killed during mowing. Therefore, it can be assumed that intensive mowing of agricultural grassland, which damages not only adults, but also larvae and nymphs, amounts to an “overfishing effect” that has significantly contributed to the current insect decline on agricultural land. In our project we are investigating how a technical modification of a standard disc mower and an effective insect flushing bar might reduce the negative effects of mowing on grassland ecosystems. Here, we present results from our first year testing a flushing bar prototype combined with a conventional disc mower. Our results show a notable scaring effect by which 45 % of insects were scared away and escaped from the mowing machine when flushing bar and mower were tested together. Further, we could detect medium-term effects of mowing regardless of the used mowing technique with 30 to 56 % less insects on mowed plots compared to untreated controls. In the light of the ongoing loss of biological diversity we intend to gain deeper knowledge on the direct, medium- and long-term effects of mowing by testing various mowing technologies and their effects on spiders, as well as important pollinators, herbivores, and predatory insects.

Diversity of Insects in Nature protected Areas (DINA) – results from four years of transdisciplinary research on insect decline

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NABU (The Nature And Biodiversity Conservation Union)

Insects provide various vital functions in ecosystems, but in recent years a dramatic decline in their biomass has been observed. As part of the transdisciplinary research project DINA, the biomass of flying insects from Malaise traps was recorded in 21 representative nature protected areas across Germany, and the species were determined by DNA metabarcoding. To understand the influences leading to their decline, various factors including pesticide and fertilizer application, as well as changes in vegetation and findings from spatial analysis were considered. To align the results with the needs of society, local workshops, and stakeholder surveys were conducted. The results of the project show clear impacts of arable farming on insects and their habitats. Contact with several pesticides, impoverishment of vegetation by fertilisers on the edge of protected areas, and long common boundaries between protected areas and arable land contribute to the documented loss of flying insects. Biomass and insect richness according to DNA metabarcoding strongly correlate, which suggests that biomass is a reliable indicator for the status of their diversity. The Ellenberg indicator value for nutrients turned out to be a reliable metric for the condition of vegetation and pointed out the loss of endangered species. However, the management of nature protected areas also shows considerable deficiencies and does not address many problems or offers hardly any risk assessment, especially for insect conservation. Recommendations based on the results and expertise of the DINA project were derived, which suggest buffer zones of 2000m around nature protected areas to strengthen the protection of insects.



Different behavioural-ecological aspects of migratory European Columbiformes and their relevance for conservation

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We analysed different but connected behavioural-ecological aspects of migratory species of Columbiformes (doves and pigeons) with breeding grounds in Europe. The species studied include one of the most rapidly declining European breeding birds, namely the European Turtle Dove (*Streptopelia turtur*). As a Palearctic-African migratory species, it is the only long-distance migrant among the European native wild Columbiformes. On the contrary, the other two studied species Common Woodpigeons (*Columba palumbus*) and Stock Doves (*Columba oenas*) are both short-distance migrants. These species are among the most common European breeding birds or have a stable population trend, respectively. Migratory and also foraging movements can affect ecological processes, such as the spread of diseases, inter- and intraspecific competition and the ability of species to cope with global as well as local environmental changes. Besides migration and foraging strategies themselves, these ecological processes can lead to differences in individual fitness, which may subsequently influence population dynamics. We present results from (a) satellite tracking projects for Turtle Doves and Woodpigeons focusing on migration and foraging patterns, (b) from an assessment of diet components based on DNA metabarcoding of faecal samples, and (c) from a study on Haemosporidia infections. The obtained results shed light on shared and divergent behavioural-ecological traits within the three species. Moreover, they indicate how the intraspecific variability in behaviour might contribute to the differing population dynamics and how improved knowledge on the behavioural-ecological aspects can help with the implementation of management and conservation measures, particularly for rapidly declining Turtle Doves.

Age-dependent perfume development in male orchid bees, *Euglossa imperialis*

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Males of the neotropical orchid bees concoct complex perfume blends by collecting volatile substances from various environmental sources, including orchids. These perfumes are stored in specialized hind-leg pouches, and are released during courtship displays, serving as inter-sexual signals. It has been proposed that male perfumes provide honest information on male fitness. If perfume traits such as quantity or complexity increase over an individual's lifetime, they could bear information about age (survival) and foraging success of males. We conducted a two-season mark-recapture study with *Euglossa imperialis* in pacific Costa Rica, monitoring the balance of perfume uptake and expenditure over the lifespan of individual males. We used superglue to seal one hind-leg pouch at first capture, thereby “freezing” the perfume status on one body side. By comparing contents to those of the pouch of the other body side upon second capture, we assessed the relative changes of perfume traits over time for individual males. Furthermore, we developed a novel method to estimate individual age by combining two parameters of wing degradation. Our results show that young to intermediate-aged individuals possess both the largest quantities of perfume and the largest number of detected compounds, contradicting the hypothesis of a cumulative increase in perfume loads over their lifetimes. While this does not generally disprove an indicator function of male perfume, it suggests that perfume load does not simply reflect age (survival) but rather encodes other non-cumulative fitness components such as orientation and navigation abilities or sensory acuteness.

Developing a biohybrid entity for aquatic environment observations

Nikolaus Helmer; Wiktoria Rajewicz; Thomas Schmickl; Ronald Thenius

University of Graz

The complexity of ecosystems makes it necessary to look at habitats from as many angles as possible. This is especially true for water bodies, since monitoring water quality and ecology is becoming increasingly important as these habitats are under growing environmental stress. Until now, monitoring of water bodies has mainly been done through water sampling and "classical" electronic sensors, e.g. for oxygen saturation, temperature and pH. In the EU project Robocoenosis, these sensors will be extended with a biohybrid component and combined with the automatic evaluation of the behavior of selected organisms (e.g. mussels and Daphnia). Since the organisms can react in real time with specific behavior to their environment, a more holistic data set on a water body will be generated. In addition, communities of organisms (e.g. plankton and algae) will be studied to document long-term developments in the water body. Furthermore, the project Robocoenosis aims to build as much of the biohybrid entity as possible out of biodegradable materials. Combined with an energy supply consisting of microbial fuel cells (MFC) and solar cells, this will enable autonomous, cost-effective and automatic monitoring of water bodies over longer periods of time. The individual components of the biohybrid entity are continuously tested for functionality in the laboratory and in the field in Austrian lakes and are already achieving runtimes of several months.

The use of *Daphnia* as a living sensor in aquatic biohybrid systems

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University of Graz

Daphnia is an important model species for studying the aquatic environment. It has a vital role in the aquatic food chain and its reactions to certain ecological contaminants, provide an estimation of their toxicity in the aquatic habitat. In the project Robocoensis, we aim to implement this approach into the field and automate behavioural observations by using a biohybrid. The developed biohybrid entity combines electronic and mechatronic components with living organisms. The artificial parts observe the organism of choice, here *Daphnia* individuals, and draw conclusions on the state of the environment from their swimming patterns. The calibration is performed in two ways: 1) monitoring the reaction of the animals to confinement and 2) laboratory-based experiments investigating *Daphnia*'s reaction to specific environmental changes. Several field experiments showed that the robotic setup constructed for *Daphnia* observation allows them to display their normal swimming, feeding and breeding behaviour and further long-term tests are planned. Laboratory analysis was performed first with the use of salt (2.5, 3 and 4.5 ppt) and will be continued with other environmentally-relevant contaminants, such as pesticides, sunscreen and others. The first experiments showed that salt causes a clear disruption in *Daphnia* swimming behaviour and causes different degrees of reaction depending on the salinity level. The behaviours were classified as "spinning", "movement inhibition" and their combination ("disrupted"). In the lowest salinity (2.5 ppt) *Daphnia* showed significantly less disrupted behaviour compared to the two higher salinity levels. The spinning and movement inhibition also varied in different salinity levels with the 2.5 ppt showing lower spinning levels and lower movement inhibition compared to 3 and 4.5 ppt. These preliminary results show that by observing the basic swimming patterns of *Daphnia* it is possible to not only detect the presence of a stressor but also its intensity in the environment.

Insights into the mechanisms of sex bias in the hybridogenetic flea beetle, *Altica lythri*

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Altica lythri is a hybridogenetic beetle with unique reproduction anomalies that provide an ideal model for understanding how genetic conflicts shape the sex, and thus evolution, of species. Ancient interspecific hybridization and Wolbachia bacterial infections in *A. lythri* resulted in introgression of mitochondrial (mt) DNA (HT1, HT2, HT3). Depending on a female's mtDNA haplotype and Wolbachia strain (HT1- wLytA1), the progeny can consist exclusively of daughters. This female bias could, on one hand, be caused by the Wolbachia infection itself, which is known to induce male-killing, feminization of genetic males and parthenogenesis. On the other hand, the female bias could also have its cause in the genetic consequences of hybridization in the form of nuclear-cytoplasmic conflicts between introgressed mtDNA and nuclear genes, which result in the absence of heterogamous males with the mtDNA HT1. To understand the mechanisms that contribute to this sex-specific bias, we have extensively studied the different mechanisms by which Wolbachia can interact with its host to manipulate the sex as well as the Wolbachia infection itself. We address these issues in *A. lythri* with genomic and molecular approaches. Using different PCR techniques, we established and applied screening methods for phenotypic and genetic sex determination in the different life stages of *Altica lythri*. Paternity analyses by a dAD approach as well as chromosome spreads of unfertilized eggs enabled us to show that the HT1 females reproduce by gynogenesis. Our results provide key insights into how sex determination is altered and whether selfish genetic elements and/or reproductive manipulating endosymbionts play the key role in this system.

Evolution of alternative reproductive systems in *Bacillus* stick insects

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Stick insects of the genus *Bacillus* feature a variety of reproductive strategies including canonical sex, female-producing parthenogenesis and hybridogenesis (elimination of the paternal genome and clonal transmission of the maternal genome). The different parthenogenetic and hybridogenetic lineages were suggested to be of interspecific hybrid origin between the same two sexual species, but it remains unknown whether they derive from independent hybridization events or whether a single event was followed by a secondary diversification of reproductive modes. Here we revisit the different species and hybrid lineages within the genus *Bacillus* using a de novo chromosome-level reference genome and RADseq data of > 500 wild-caught individuals. We confirm that two hybridogenetic lineages and one parthenogenetic lineage show the genomic signatures of a diploid F1 hybrid species with the maternal genome from the sexual species *B. rossius* and the paternal genome from the sexual species *B. grandii*. A second parthenogenetic lineage shows the genomic signature of an allo-triploid, derived from three parental species, *B. rossius*, *B. grandii* and *B. atticus*. We then phased the parental haplotypes in each hybrid lineage to infer their phylogenetic relationships and study the origin of different reproductive modes. Our phylogenomic analyses suggest that sex was lost after a single hybridization event followed by at least one secondary reproductive mode diversification, most likely a transition from hybridogenesis to parthenogenesis and triploidy. These findings indicate transitions between unorthodox modes of reproduction that were previously not known to occur, suggesting that the loss of sex per se can be a driver of reproductive mode diversification.

One clone to rule them all: population genomics of the water flea *Daphnia* in Arctic lakes (SW Greenland)

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Arctic regions including Greenland have strongly been impacted by climate change in the past 50 years, as well as across the past centuries. Freshwater systems are among the most affected environments, resulting in shifts in temperature as well as in the duration of ice cover periods that lead to profound changes in the environment. They affect the length of the growing season, light conditions, and lake productivity among a variety of other environmental conditions. Arctic lakes and ponds often harbour populations of the cladoceran *Daphnia* that are polyploid, a trait that is more frequently encountered in higher latitudes or altitudes. Polyploidy in *Daphnia* is generally associated with obligate asexuality, and the resulting clonal lineages are thought to be relatively shortlived with an estimated age of only decades. Here, we studied the genetic composition of *Daphnia* populations from several lakes near Kangerlussuaq, West Greenland. We collected dormant eggs from dated sediment covering the last two to three centuries, using a whole genome sequencing approach. All populations were found to be triploid, obligate asexuals. Within-lake genome diversity was extremely low with one clone dominating each population across the entire time span studied. Possible implications for evolutionary adaptation and fate of these populations in the light of climate change will be discussed.

Phenotypic evolution of Arctic *Daphnia* linked to environmental change?

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The ongoing anthropogenic environmental change can pose immense challenges to organisms and their ecosystems. Environmental change in the Arctic has been advancing at an accelerated pace compared to the rest of the globe. Thus, Arctic ecosystems have faced important changes in various conditions such as temperature and precipitation in the past decades. Studying how organisms might have already adapted to the new conditions could help understand ongoing and future microevolutionary responses, shedding some light to the future of populations and ecosystems. Resurrection ecology is useful for studying evolutionary history using resurrected cysts or eggs deposited in lake sediment. This method grants access to phenotypic traits of past organisms exceeding information on morphology and genomes, such as their physiological responses. One of the organisms ideal for such studies is *Daphnia*, a freshwater cladoceran keystone species. One of its advantages is the possibility to keep asexual lineages in the lab, which allows testing multiple traits on the same lineage. In this study, we hatched 50-year-old *Daphnia pulex* from eggs dormant in the sediment of an Arctic lake (Braya Sø, South-West Greenland). Furthermore, we obtained adult animals from the current population of the same lake to set up lab cultures. Previous work indicated that this population has consisted of a single asexual clone for at least 200 years. Using multiple lineages from each temporal subpopulation we compared their respiration rate and upper thermal limit measured as time to immobilization (Timm). We found a significant difference in phenotypic responses between- but limited variation within- the two temporal subpopulations. Our results suggest phenotypic evolution in the Braya Sø *Daphnia* population, which may be linked to environmental change.

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 – but this shift has occurred more rapidly than climate has changed. Recent investigations showed that early instar spiders (spiderlings) from edge populations tolerate colder temperatures better, strongly suggesting local adaptation to colder winter conditions. Nevertheless, edge spiderlings weigh less than core spiderlings, and all lose weight during warm winter treatments. 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 the effects of temperature on 1) survival, 2) lipid content, 3) metabolomics, and 4) gene expression. The spiderlings of this cold-adapted population showed a high degree of temperature tolerance, as the survival rate and overall lipid content were not affected by the different winter conditions. The same pattern can be observed for saturated and monounsaturated fatty acids. However, the Omega 3 polyunsaturated fatty acids (PUFAs) are lower in spiders that experienced warm winters, likely due to higher activity. Spiderlings from the warmer treatment might be at a disadvantage after emergence from the egg sac in spring due to lower reserves of these Omega 3 PUFAs. We utilize a high-quality reference genome and metabolomics data focusing on differential expression of cell repair genes, heat shock proteins, and cryoprotectants to help us understand the responses to different winter temperature regimes. This information on the degree and mechanisms of plasticity can help us predict the responses of recently cold-adapted populations to climate change.

HSP90 as an evolutionary capacitor in *Tribolium castaneum*

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Evolutionary capacitance is the process by which populations ‘store’ genetic variants in the form of cryptic genetic variation (CGV) and then release them under stressful environmental conditions. As this may lead to a subsequent assimilation of potentially adaptive phenotypes, this process could enhance evolutionary adaptation. In the model organism *Tribolium castaneum* (red flour beetle), we previously found that experimental reduction of HSP90, a molecular chaperone which is known to be an important evolutionary capacitor, repeatedly led to assimilation of a reduced eye phenotype (RE). It was further shown to have fitness benefits in terms of higher number of offspring. Since the RE phenotype affects the light-receiving organ, we studied if this phenotype influences the light-entrained circadian activity patterns using automated activity tracking of individual beetles. We found that the RE beetles had a lower startling response to the morning light indicating a difference in response to light. This suggests that the newly emerged phenotype could also lead to behavioural differences in the population. A study in *Drosophila* had shown that experimental reduction of HSP90 can directly contribute to behavioural variations possibly through the capacitance function. To study a potential role of HSP90 as a capacitor of behavioural variations in *T. castaneum*, we monitored locomotor activity patterns directly upon HSP90 inhibition. We observed differences in the activity profiles and circadian rhythmicity in comparison to control beetles. Our study provides experimental tests for the role of evolutionary capacitance in contributing to more variations and thereby potentially speeding up the process of adaptation.

Interrelation of circalunar, circadian and photoperiodic time keeping in the marine midge *Clunio*

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Marine midges of the genus *Clunio* inhabit the intertidal zone along rocky coasts around the world. *Clunio*'s life cycle is adapted to the tides. Adults of *Clunio* only emerge and reproduce during full moon and new moon, when the spring tides expose most of the intertidal zone. To this end, *Clunio* evolved endogenous time keeping mechanisms. These include the circalunar clock, which restricts adult emergence to spring tide days, and the circadian clock, which restricts adult emergence to the time of low tide. We investigated the role of the circadian clock in circalunar time keeping by exposing midges to non-24h day cycles. The observed linear relationship between daylength and the circalunar clock's period suggests that *Clunio* is counting days, or rather circadian oscillations, in order to measure the lunar cycle. We now further explore if *Clunio*'s circalunar counter is derived from a photoperiodic counter using comparative transcriptomics. In an evolutionary perspective, the circalunar clock mechanism of *Clunio* and another marine midge appears to be distinct from those of marine algae and annelids.

How moonlight sets the circalunar clock in *Clunio marinus*

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Many organisms inhabiting the interface between land and sea have evolved biological clocks corresponding to the semilunar (14.77d) or the lunar (29.53d) cycle. Since tidal amplitude is modulated across the lunar cycle, these circasemilunar or circalunar clocks help organisms to adapt to the tides. Biological clocks are synchronized to external cycles via environmental cues called zeitgebers. Moonlight as a zeitgeber for circalunar rhythms started to be explored in the 1960s but many questions remain open. Here, we explore how light at night sets the circalunar and circasemilunar clock of *Clunio marinus*, a marine insect that relies on these clocks to control timing of emergence. We first characterized how moonlight intensity is modulated by the tides by measuring light intensity in the natural habitat of *C. marinus*. In laboratory experiments, we then explored how different moonlight treatments set the phase of the clocks of two *C. marinus* strains. Light intensity alone does not affect the strength or phase of the lunar rhythm. Presenting moonlight during different 2-hour or 4-hour windows during their subjective night shows that (1) the required duration of moonlight is strain-specific, (2) there are strain-specific moonlight sensitivity windows and (3) timing of moonlight can shift the lunar rhythm in a way that it stays in tune with the lowest tides. Experiments simulating natural moonlight patterns with moonrise and moonset confirm that the phase is set by the sensitivity windows and that natural light intensity leads to the best synchronization. Taken together, we show that there is a complex and strain-specific integration of light intensity, duration and timing of light at night to precisely set the strength and phase. The observed fine-tuning of the lunar rhythm under the most natural moonlight regimes lays the foundation for a better chronobiological and genetic dissection of the circa(semi)lunar clocks in *C. marinus*.

Evolutionary change in the benefit of parental care

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Parental care is a taxonomically widespread strategy that has evolved because it increases offspring fitness. However, the benefits of care may change over evolutionary time, as parent-offspring co-adaptation or reduced maternal investment in the developing embryo may increase offspring dependence on care. Therefore, assessing the current adaptive benefits of parental care may provide little insight into such benefits during its initial evolution from an ancestral state of 'no care'. Moreover, there have been few attempts to understand changes in the benefit of care in the evolutionary history of a lineage. Carrion beetles vary widely in the extent of parental care, from no care at all to the highly advanced care of burying beetles (genus *Nicrophorus*), where parents regurgitate predigested food directly to their offspring. We conducted a phylogenetically controlled, common-garden study of 17 carrion species across the full spectrum of parental care. In all 13 *Nicrophorus* species we studied, parental presence accelerated offspring growth and growth rates were faster than in any of the non-caring species. However, the impact of care on offspring survival differed greatly among species, with a clear phylogenetic signal in the survival benefit of care. Our analysis suggests that once parental care was established, offspring evolved to become highly dependent; however, this trend was later reversed, resulting in offspring of some species becoming largely independent again. In burying beetle species, the degree of larval dependence was not related to maternal investment in egg size, nor to larval growth rate or mean brood size. However, in more dependent species, larval begging rates were higher than in species with less dependent larvae. Our study illustrates how the benefits of care can evolve dynamically, with the potential for either an increase or decrease of the benefits over time.

Immune priming: causes and implications for pathogen evolution in the model organism *Tribolium castaneum*

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Invertebrates lack the cellular machinery that is needed to provide adaptive immunity. However, over the past decades it was shown that many members survive an infection better if they encountered the corresponding pathogen or its pathogen-derived cues before, a phenomenon called immune priming. How are these memory-like responses established? And what kind of selection pressure does immune priming impose on the pathogen? We use the model organism *Tribolium castaneum* (Tc) and its pathogen *Bacillus thuringiensis tenebrionis* (Btt) to investigate these questions. When fed with sterile supernatant of a grown Btt culture mixed with flour, larvae of Tc survive a subsequent infection with Btt spores better, even up to 4 days after being exposed to the “priming” diet. We investigated the proteome of the supernatant that is used for the “priming” diet, observed morphological changes in the guts of primed larvae compared to non-primed larvae and studied the infection dynamics in Tc larvae when exposed to Btt spores by comparing the Btt 16S rRNA levels in primed and non-primed larvae. We further performed experimental evolution of Btt in primed and non-primed hosts to analyze both, phenotypic changes such as spore load and virulence, as well as genomic changes by sequencing whole genomes of the evolved pathogen lines using PacBio long-read technology. This work is an example for how to combine questions about the mechanistic underpinnings of a trait or phenomenon with insights gained from experimental evolution studies.

Microbiome structure of dysbiotic and symbiotic associations of fungus-gardening ants

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One of the most important evolutionary transitions is the cooperation of vastly unrelated species (symbiosis). What stabilizes symbiotic associates or causes their collapse (dysbiosis) are often unknown yet important. Fungus-gardening ('attine') ants are excellent model systems for exploring how symbiosis exhibit stability as the host and symbionts are macroscopic and can be easily experimentally manipulated. We explored whether changing fungal partners impacts the microbiomes of the host ants and their symbiotic fungus by performing cross fostering experiments that forced ants to grow novel fungi. Prior work has demonstrated that some of these combinations are known to be unstable. As a result, we were able to compare microbiomes that lead to symbiosis and those that lead to dysbiosis. The experiments revealed that fungus-gardening ants alter their novel garden microbiomes into configurations that are similar to those found in nature and these produce unstable communities with respect to certain clades of cultivated fungi. Though the exact mechanisms have not yet been determined, since the bacteria that exhibit the most change are involved in nutrient cycling, it would appear that the adaptiveness of certain ant-fungal-bacterial combinations has a nutrition function rather than some other mechanism, such as defense.

How differing functional demands of mouthparts and sensory systems determine head shape and modularity in an insect

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In many insects, mandibles are used to shear and crush food items. This is often achieved via some degree of asymmetry between the mandibles, allowing crossing and occlusion. In the grasshopper *Schistocerca gregaria*, this asymmetry is strictly directional, the left mandible always being larger and overlapping the right one. However, it remained unclear whether this asymmetry impacts bite force as well as the shape variation of other head parts. In particular, sensory systems such as the eyes should be selected for symmetry which would counter asymmetry from the mandibles. Consequently, modularity signal should show some degree of independence between ventral and dorsal head parts. Here, we use 3D geometric morphometrics and bite force measurements to decompose the different types of asymmetries across the head and mandibles and test for their relation with modularity and integration signal as well as bite force. Our results show that the best supported modularity partition for the grasshopper head has two modules: i) the head capsule, tentorium and sensory structures as a whole, and ii) both mandibles combined, which are at the same time strongly integrated. This pattern matches the symmetry-asymmetry patterns observed, whereby the head structures are generally symmetric, contrary to the mandibular structures which are directionally asymmetric. Finally, contrary to our expectations, we show that the magnitude of asymmetry across the head, or in the mandibles only, does not impact bite force performance. Our results show that in an organ such as the head, harboring both sensory systems selected for symmetry and mouthparts selected for asymmetry, there is a symmetry gradient from dorsal to ventral for the head capsule. Modularity was certainly key in the evolution of such gradient, by allowing the head capsule and mandibles to respond independently to functionally divergent selective pressures.

Measurement and Analysis of Animal Closing Forces: Unlocking Insights into Micro- and Macroevo

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Bite and pinch forces in animals are crucial to access food and compete for mates. As such, they are performance metrics linked to an individual's fitness. However, our understanding of the tempo and mode of bite force evolution, especially in insects, remained limited due to their small size. We introduce a novel, mobile, and lightweight force measurement setup specifically designed to quantify in vivo closing forces in a wide range of animals, with a particular focus on smaller taxa. The setup employs a highly sensitive piezoelectric force transducer, converting the forces into electrical signals, which are then amplified using a custom charge amplifier. The resulting data allows for the extraction of maximum force values and individual force curves, providing insights into the underlying biomechanics, physiology, and behaviour during biting and pinching actions. By offering a readily accessible and reproducible measurement solution, we anticipate that our novel setup, along with the accompanying R package, will facilitate the study of animal closing forces in both field and laboratory settings. We used our new device and R package to measure and analyse the macroevolution of bite force and bite curve shapes in roughly 650 insect species that range between 0.3 to 18 cm. From this dataset, surprising evolutionary patterns of allometry and taxonomic constraints emerged and were compared to the data available from vertebrates. Our new integrated approach has the potential to advance research on the micro- and macroevolution of closing forces across a diverse range of taxa, particularly in the highly diverse arthropods and smaller vertebrates.

The Visual Systems of Crab Spiders and Running Crab Spiders: convergent or conserved?

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Despite over 100 million years of divergence (Shao and Li, 2018), the spider families Thomisidae (crab spiders) and Philooidae (running crab spiders) exhibit remarkably similar body morphologies, possessing flattened bodies and elongated front legs (Wolff et al. 2022), and ecologies, being visually guided ambush predators. This is evidenced by their characteristic widely-separated eyes and well-developed visual neuropils (Long et al. 2021). However, whether the visual systems in the two families are similar, and if so, whether they are convergently evolved or ancestrally similar remains largely unknown. Here, we used geometric-morphometrics (GMM) on synchrotron micro-computed tomography (SR μ -CT) scans of spider cephalothoraces to compare the position, arrangement, and sizes of eyes in 20 species representing the two families, and 4 species from families phylogenetically separating them. Our results provide a foundation and framework for further comparative studies on arrangement and position of eyes across spider families with varying ecologies.

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Evolutionary genetics of convergent mimicry color patterns in bumblebees

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Convergent evolution of complex phenotypes is one of the most 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 (Muellerian mimicry). However, the molecular underpinning of these striking case of evolutionary convergence are not well understood except since recently in some butterflies. A group of important pollinators, bumblebees, have repeatedly 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 repeated convergent evolution. We will provide insights in the genetic mechanisms facilitating color shifts in bumblebees, but also whether repeated convergent shifts are underpinned by the same mechanisms, or stem from different molecular changes. Here, we are presenting a population genomics study on three bumblebee species showing how repeated evolution of phenotypic transitions is reflected on a genomic scale.

Evidence for Incipient Allochronic Divergence Over the Lunar Cycle in an Intertidal Midge

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Populations that time their reproduction through heritable mechanisms have the potential to speciate through allochrony, i.e. the separation of reproduction in time between individuals. While a number of studies have shown allochronic divergence to happen in wild populations over the seasonal cycle, it is still unknown if this process can take place over shorter cycles like the lunar cycle. Here, we investigate the sympatric divergence of chronotypes (i.e., subpopulations with different timing phenotypes) of the marine midge *Clunio marinus*, which times its development and reproduction to the lunar cycle. We first assess the effectiveness of differential reproductive timing to identify potential sources of gene flow between chronotypes. Then we investigate a number of potential barriers to reproduction using a data set of wild and laboratory crosses, but cannot find any evidence of gametic incompatibilities, hybrid breakdown or hybrid sterility. Additionally, there is also no evidence of assortative mating beyond differences in timing. We conclude that allochrony alone maintains reproductive isolation between chronotypes, though it is an imperfect barrier to reproduction. Furthermore, we surmise that divergence must have been even by allochrony, and likely happened relatively recently. Thus, these sympatric chronotypes of *C. marinus* represent the first example of incipient allochronic divergence over the lunar cycle.

Support for a radiation of free-living flatworms in Lake Tanganyika

Jeremias N. Brand

The African Great Lakes have long been recognized as an excellent location to study speciation. Most famously, cichlid fishes have radiated in Lake Tanganyika and subsequently spread into Lake Malawi and Lake Victoria, where they again radiated. Other taxa have diversified in these lakes, such as catfish, ostracods, gastropods, and Monegenean gill parasites of cichlids. However, these radiations have received less attention and the process leading to their speciation in this unique region remains to be further explored. Here I describe a radiation of *Macrostomum* flatworms that offers a good opportunity to conduct such investigations. Recent field work has revealed a monophyletic clade of 16 *Macrostomum* flatworms that have, to date, only been collected from Lake Tanganyika. Additionally, a species collected from Lake Malawi was found nested within this clade. Molecular phylogenetic analysis, largely based on transcriptome data, suggests that this clade underwent rapid speciation, possibly due to a large habitat diversity in the Lake. I also observed significant differences in the sperm morphology of these flatworms compared to those of species found outside the Lake Tanganyika and Lake Malawi. These included the elongation of an anterior structure, a reduction in the size of the lateral sperm bristles, and changes in the relative proportions. These findings support the hypothesis that a clade of *Macrostomum* flatworms has radiated in Lake Tanganyika and subsequently spread to Lake Malawi. Therefore, the African Great Lakes are promising targets for further research into flatworm diversity and speciation.

Decoding the multimodal courtship signal of a parasitoid wasp

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Courtship displays are typically multimodal, so they include at least two signal components which are transmitted and perceived over different sensory channels. However, most past studies on multimodal courtship displays have been focusing on isolated signals or signal specialists, thereby falling short of explaining why these multimodal displays evolved besides the additional costs involved. Males of the parasitoid wasp *Leptopilina heterotoma* (a parasitoid of drosophila) show a complex courtship behaviour consisting of wing fanning and antennal stroking. While the wing fanning produces a vibrational signal, the antennal stroking most likely transfers a species-specific antennae pheromone. Here, we test the hypothesis that the two signal modalities correspond to the species recognition hypothesis, i.e., one modality contains information on the species identity while the other modality informs about the sender's quality. To determine which display component transmits mate assessment information, we conducted mating trials with unmated couples of *L. heterotoma*, during which we recorded the male's wing fanning frequency. After the trial we measured the body size and analysed the individual chemical profile of each male. We found that the male mating success was positively influenced by the fundamental wing fanning frequency and body size, but the chemical profile did not show significant differences between successful and unsuccessful males. Our findings thus indicate that the vibrational display component conveys mate assessment information, whereas the chemical display component functions as additional species recognition mechanism, confirming our hypothesis that the *L. heterotoma* courtship display is an example of the species recognition hypothesis. Overall, our data give novel insight into the information content of multimodal signals in insects and provide a basis for future studies to fully understand their function and evolution.

The evolution of honest and dishonest signals of fighting ability

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Weapons and body size play an important role in determining the outcome of contests over resources, and may also serve as reliable cues of an individual's fighting ability. In contrast, other traits may dishonestly exaggerate an individual's fighting ability in order to intimidate opponents into retreating. Such signals potentially evolve via very different mechanisms than the signals used in mate choice, yet their evolution is still poorly understood. We contrast the evolution of honest and dishonest signals of fighting ability using a game-theoretic model. Our model predicts the coevolution of body size, an honest or dishonest signal, and an individual's tendency to escalate aggressive interactions. The results reveal that both honest and dishonest signals can exaggerate far beyond their ecological optima, but that exaggeration is more pronounced for honest signals. Whether a signal is honest or dishonest does not strongly affect the tendency to escalate aggressive interactions.



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

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During their development many insects undergo ecological niche shifts, often requiring functional adaptation. Here, we quantitatively characterized morphological shape differences in an ontogenetic series of the head of the dragonfly *Libellula depressa* (Odonata: Libellulidae) using landmark-based geometric morphometrics. In total, 107 specimens across all twelve larval stages were studied using a set of landmarks and semilandmarks (132 individual points). The landmark datasets were aligned using a novel script that rotates the landmarks associated with movable mouthparts into a common position (i.e. standardized joint angles). This allowed to correctly perform a subsequent generalized Procrustes superimposition of the full landmark sets. Our results show that both for the head as a whole and its individual parts (with their respective centroid sizes), a significant allometric relationship is present. The disparity between individuals from larval stage one is significantly larger when compared to that of all other stages. The disparity within larval stages is considerably large on Principal Components (PCs) 1 and 2, up until stage six, after which the distance between individual datapoints decreases. This suggests diminishing morphological variance after stage six. We show that after allometric correction, the observed shape variance does not follow a specific pattern along the PCs. Therefore, it is likely that considerable proportions of ontogenetic shape variance in the larval dragonfly head are due to differences in size. The results obtained in our study suggest that the shape of the head and mouthparts in larval dragonflies might be relatively conserved during ontogeny, implying low plasticity of function. Morphometric studies of changes in the insect head over an ontogenetic trajectory are scarce – therefore, our work represents a significant step forward in understanding the relationships between characters like shape, size and function.

Successful crab invader in the Kiel Canal - The secret lies in reproductive biology

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The mud crab *Rhithropanopeus harrisii* (Gould, 1841) [Panopeidae, Crustacea], commonly known as the Zuiderzee crab, is one of the most widely distributed crab species worldwide. It is native to the eastern Pacific Ocean in North America. It invaded the Kiel Canal in 1936 and has successfully reproduced ever since. 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 transport of sperm, the interaction between the male gonopods with the female reproductive organs, and egg fertilization. In addition, the reproduction cycle needs to be studied in more detail in order to understand the development of juvenile crabs, the onset of reproductive capacity, and any changes in the tissues of the reproductive organs and, consequently, a change in their function. Using histological and morphological methods, we reconstructed the inner organs of the female and analyzed the reproduction cycle of *Rhithropanopeus harrisii* to explain the fertilization mechanisms of the eggs and their potential advantage in terms of the timing of their reproduction capacity. These results were compared to morphological data of the reproductive systems of other brachyuran crabs and to the reproductive cycle of other Baltic Sea species. Understanding the functional morphology of the reproductive organs and the timing of the reproduction cycle of *Rhithropanopeus harrisii* in the Kiel Canal may contribute to a better understanding of the successful invasion process of this crab species.



The structure and development of the trophosome in the frenulates *Siboglinum fiordicum* and *Oligobrachia* sp

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Frenulate pogonophorans (Annelida: Siboglinidae: Frenulata) are tubeworms that inhabit reduced biotopes, such as methane seeps, the periphery of hydrothermal vents, and sediments rich in organic matter. Frenulates are the sister group to all other siboglinids, which all are gutless worms that are depending on the symbiosis with bacteria. The trophosome is a specialized organ that is housing their intracellular symbionts. In the frenulate *Siboglinum fiordicum*, this organ develops during the transition of the competent larvae into the adult. It occupies a large part of the body volume in adult worms, like *S. fiordicum* and *Oligobrachia* sp. It is a cylindrical organ with lumen whose is surrounded by two layers, an external and internal epithelium. Cells of the external layer contain storage granules with glycogen, lipids. The cells of the inner epithelium encompass the symbiotic bacteria. We show that during the life cycle the bacteriocytes with the symbionts migrate from the anterior to the posterior end of the trophosome. With the use of fluorescent in situ hybridization we show the distribution of bacteria within the cells of the trophosome. Based on our ultrastructural and histological studies of larvae, juveniles and adults, we hypothesize that the trophosome in frenulates develops from the endoderm. This is in contrast to previous studies on other siboglinids (vestimentiferans, bone-eating worms, and wood-borers), where the trophosome develops from the mesoderm. It remains unclear if the trophosome of Frenulata originated convergently in evolution, or if their developmental origin of a homologous trophosome changed. Further studies of the trophosome development in more siboglinids are needed to resolve this question.

On the springtail jumping: a detailed and comparative morphological study of the jumping apparatus

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The internal and external morphology of the jumping apparatus of a springtail will be presented in detail. A morphological comparison will be made between different taxa of Collembola, e.g. Entomobryomorpha, Neelipleona, Symphypleona and Poduromorpha. Morphological convergent patterns of the jumping apparatus will be shown and related to ecological aspects. The morphological structures of the jumping apparatus, for example the furca (catapult structure), retinaculum (latching structure), and elastic basal plates (potential spring structures) will be discussed in detail as well as their potential functions. A narrative on evolutionary and morphofunctional aspects will be build. A warm discussion is welcome, I bring the wood and you can start the fire.

Fluid origin, its physical properties and interactions with the substrate in the tarsal attachment pads of stick insects (Phasmatodea)

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In insects, attachment is facilitated by different adhesive systems, which generate attachment through maximization of the contact area with the substrate. In stick insects, this is facilitated by relatively large and smooth cuticular pretarsal (arolia) and tarsal (euplantulae) attachment pads. To support this process, stick insects secrete a tarsal fluid into the interface of the attachment pad and substrate, which aids in adaptation to different substrate features, such as roughness, surface energy and contamination. Even though a lot is known about the composition of the tarsal fluids in insects, information regarding the interplay between the fluid and the substrate surface is scarce, especially in stick insects. This includes its production, the intermediate storage within the adhesive pad, the transport to the surface of the pad, as well as the morphological and evaporation characteristics of the fluid on the substrate.

To analyze the inner morphology and materials of the adhesive pads, focusing on the transportation pathway and some physical characteristics of the tarsal fluid, we utilized cryo-scanning electron microscopy (cryo-SEM), confocal laser scanning microscopy (CLSM) and histological methods. To assess the fluid's evaporation rate and morphological appearance, we applied cryo-SEM and white light interferometry (WLI). Our observations suggest that the tarsal fluid is produced by exocrine epidermal cells and transmitted first into a spongy layer and then into the interspaces between the procuticle fibril arrays of the attachment pads, which serve as an internal reservoir. While inside the attachment pad, the fluid in combination with cuticle material can potentially contribute to the viscous-elastic properties of the pad. When applied to the substrate, it differentiates into four morphologically distinct components and exhibits different evaporation rates.



Larval aggregation of Erythraeidae mites in about 100 million years old Myanmar Amber: Seeking a host?

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The six-legged larvae of the protostigmatan mite ingroup Parasitengona are known for parasitising insects (and other representatives of Euarthropoda). They externally attach to their hosts by their mouthparts. This behaviour is also known in larvae of the ingroup Erythraeidae. For a better understanding of the evolution and diversification of this behavioural strategy, fossils can provide important insight. Larvae of Erythraeidae have been reported in different types of ambers including cases of larvae attached to a host. We here report a piece of about 100 million years old Kachin amber, Myanmar. The piece includes 12 specimens of mites in the surroundings of an insect. The insect can be identified as an adult earwig (Dermaptera). One of the mite specimens is located near the leg of the earwig. Although many diagnostic characters of the mites are unfortunately not accessible in the amber piece, these mite larvae have general habitus that is compatible with that of many larvae of Erythraeidae: proximal parts of leg I-II separated, long legs with respect to their trunk, and the presence of a tibial claw in the pedipalps (thumb-claw process). There are numerous records of several larvae of Erythraeid attached to a single host. Thus, it seems possible that the aggregation of parasitic larvae around an insect can be explained by them being in the process of searching and attaching to their potential host. There are no previous records of earwigs being parasitised by larvae of Erythraeidae, although there are numerous reported associations between earwigs and other mite groups in the extant fauna. While larvae of Erythraeidae seem to have no preferential body part as an attachment site, legs are one of the possible locations where these mites are found on their hosts. Therefore, the mite specimen found near the leg is indicative of its potential attachment site.



Wood boring beetle larvae and the study of plant-parasitism in deep time

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Parasitism is an association between two organisms, where one organism benefits from the situation and the other suffers negative consequences. The term 'parasitism' is often used for a metazoan-metazoan interaction. However, a better understanding of associations due to new methods and technology does question whether the term 'parasitism' should comprise more than just this type of interaction. Does an organism that feeds upon a plant and weakens it also not lead a parasitic lifestyle? The group Nematoda is a rare case in which some animals have been considered to represent 'plant parasites'

So what makes an organism a parasite? Parasites live on or in the host, mostly for an extended period, and if they are endoparasitic the host provides safety from the outer environment. Parasites will increase their fitness by temporarily or permanently exploiting the host for resources, which results in the reduction of host fitness. They often show a high rate of simplification and specialization of characters. For example, they can completely lose legs and have vermiform bodies with often thickened integuments, specialized setae, and structures for attaching to a host. Many of the above-listed characters are also present in true wood borers.

The wood-boring way of life is not a new lifestyle, but a relatively old one, as supported by the fossilized trunks with bored tunnels from the Permian. Only recently various wood-boring larvae from different ambers have been reported, demonstrating how common different types of wood-boring were already in the past. At least from the Cretaceous onwards, this kind of lifestyle was apparently well-established.

Here, we discuss which aspects of wood boring support an interpretation of plant parasitism. We also show differences and similarities between fossil wood-borer larvae and animals parasitizing other animals.

Lousy attachment? – The exceptional attachment ability of the seal louse, *Echinophthirius horridus*, on marine mammals

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The seal louse, *Echinophthirius horridus*, is a unique insect parasite that infests phocid seals like harbor seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) in the North and Baltic Sea of Germany. Thereby, the marine environment poses challenges to the seal louse, such as fluctuating temperatures, high hyostatic pressure, hypoxia, and extreme ag forces. Since loss of contact with the host would mean certain death of the parasite in the open sea, a secure grip on the seal's fur is crucial for the survival of the insect. Its grip on the seal's fur is facilitated by specialized leg structures that include a modified snap hook system with claws and soft pads, allowing the louse to tightly grip the seal's hair and increase friction in sliding direction along the hair. Using confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM), we studied leg morphology and cuticle composition of the louse. We also measured attachment forces of the louse on seal fur underwater and revealed the highest attachment forces in relation to the body mass ever recorded for an insect. Safety factors, representing the attachment force per body weight, average around 4500 and reach a maximum of 18000. These findings emphasize the strong adaptation of the seal louse to the challenging ectoparasitic lifestyle in the marine environment. The results also provide valuable insights into the selective pressures arising from host-parasite interactions between marine mammals and their insect parasites.



How do spiders smell: mystery solved?

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Olfaction, the sense of smell, plays a pivotal role in the survival and reproductive success of most species. In most arthropods, olfaction is performed by cuticular wall-pore sensilla. Spiders, however, seem to lack such wall-pore sensilla. Despite the wealth of behavioral observations demonstrating the long-distance attraction of males to signaling females, how spiders smell has largely remained a mystery.

We employed field emission scanning electron microscopy (FE-SEM) and transmission electron microscopy (TEM), along with behavioral observations with a high-speed camera, to revisit the spiders' sensory equipment. We focused on the orb-web spider *Argiope bruennichi* in this study due to substantial knowledge about their reproductive biology and sensory anatomy. Behavioral observations in various contexts showed that the distal podomeres of the walking legs touch the substrate, but the proximal podomeres do not. We, therefore, hypothesized that the sensory equipment responsible for olfaction is found on the proximal podomeres. Using FE-SEM, we analyzed the morphology and distribution of chemosensory sensilla across the podomeres of male *A. bruennichi*. Using this high-resolution method, we discovered wall-pore sensilla, mainly on the non-contact podomeres. Further TEM investigation on the internal anatomy of these wall-pore sensilla revealed that they contain 1-4 chemoreceptive denites connected to the external environment by the cuticular pores.

Electrophysiological investigations targeting the wall-pore sensilla to assess their response to olfactory stimuli and a species-specific pheromone are ongoing simultaneously with the study presented here. In addition, we employ functional assays and genomic and transcriptomic approaches to identify olfactory receptor genes and receptor proteins. We expect differential expression across podomeres and between males and females.

Comparative study of the central nervous system in *Fritillaria* (Appendicularia, Tunicata)

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Tunicata, the potential sister group to Craniota, contains approximately 3000 diverse species. A distinctive trait setting the appendicularians apart from all other tunicates is the discrete trunk and tail in adult individuals as well as the specialized cellulose containing tunic, the “house” with which the animals collect and concentrate food particles. Although morphological and behavioral differences between the three appendicularian families: Oikopleuridae, Kowalevskiidae and Fritillariidae exist, most of the knowledge about appendicularians is derived from the genus *Oikopleura* and therein from the species *Oikopleura dioica*. In the genus *Oikopleura*, the house expands once after its synthesis, it surrounds the whole animal, and it is frequently discarded. In *Fritillaria*, the house expands on the anterior part only and the animal is capable of deflating and re-inflating the house at regular intervals. Therefore, in spite of the small number of cells in adult individuals, appendicularians display a variety of complex behaviors, suited to feeding, swimming, and reproduction in the water column. We present the first anatomical study on the nervous system of fritillariid appendicularians, showing detailed 3D reconstructions of the brain in *Fritillaria pellucida* as well as in *Fritillaria formica tuberculata*. Using light microscopy, serial sectioning, and 3D modeling, we describe the general anatomy, number of nerves and nuclei, as well as innervation patterns in the brain of both fritillariid species. In both species the brain consists of approximately 40 cells. A sensory vesicle is situated in the anterior region of the brain compared to a posterior position in oikopleurids. From the posterior end of the brain numerous nerves exit the lateral right side making contact with cells of the peripharyngeal band. The anatomy of the brain and nerves in both *Fritillaria pellucida* and *Fritillaria formica tuberculata* can be interpreted as adaptations to the divergent use of the house and resulting lifestyle.



The genomic and neural constituents of ant olfaction in the light of social complexity

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Olfactory perception is an essential trait for most animals, as it is involved in many vital behaviors such as mating and foraging. Odorant receptors (ORs) are one of the main types of olfactory receptors and constitute a rapidly evolving and highly diverse gene family.

Within insects, the number of ORs varies considerably and is especially increased in ants, where chemical communication shapes the cohesion of social insect societies. The expansion of the OR gene family in ants is thus suspected to be associated with the evolution of sociality and social complexity e.g., nestmate recognition and division of labor in the colony. Neuroanatomical studies further suggest that the OR family expansion is responsible for an increase of the olfactory processing system in the ant brain, as the number of glomeruli in the antennal lobes is correlated with the number of functional ORs expressed in the antennae.

To study the interplay of antennal lobe complexity and OR diversification in ants, we combine microCT scans with comparative genomics across 68 ant species from seven different subfamilies. By correlating the expansion of the OR gene family and the emerging changes in the antennal lobes of ants, with regard to antennal lobe volume, glomeruli count, and glomeruli volume, we test whether changes in social complexity (e.g., larger colonies, larger worker polymorphism, etc.) are associated with changes in the olfactory system at both the genomic and neuroanatomical level in ants.



Morphological changes of the cranial musculoskeletal system during the evolution of new trophic modes in South American Killifishes (Austrolebias)

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The evolution of new trophic specializations is often accompanied by a variety of metabolic, behavioral, and morphological changes that enable animals to explore various new habitats. One extreme case is the exploitation of seasonal ponds by annual killifishes (Aplocheiloidei). Some aplocheiloids have evolved a new life history that includes desiccation-resistant, diapausing eggs enabling them to inhabit ephemeral water bodies that cannot be exploited by non-annual fishes. The lack of other competitive fish species may have resulted in the evolution of specializations in closely related aplocheiloid species enabling to exploit different food sources. One example is the South American genus *Austrolebias*. Ancestral *Austrolebias* species exhibit a generalistic invertivorous trophic mode, preying on aquatic insect larvae, crustaceans, and small gastropods. From this mode, specialists have evolved that include substrate sifting, molluscivorous, and ichthyovorous species. Here we use a combination of histological sectioning, clearing and staining, μ CT-based 3D reconstruction, morphometry, and anatomical network analysis (AnNA) to describe and compare the cranial musculoskeletal system of *Austrolebias* species that evolved different trophic specializations. While the insertion sites of the musculature appears relatively conserved their relative volumes differ greatly. Branchial muscles associated with the pharyngeal jaw apparatus are hypertrophied in molluscivorous species while mandibular muscles are hypertrophied in ichthyovorous species showing a profound oral jaw elongation. Further AnNA reveals almost identical integration of the single skull bones within the different species. This allows asking the question if evolutionary more conserved muscles and bones correlate with a higher degree of anatomical integration.



Variation in mimicry accuracy: evolution and trade-offs in ant-mimicking spiders

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Mimicry is a classic example of the convergent evolution of traits and theory predicts that selection should favour accurate mimicry. However, while many mimics are strikingly accurate, others bear only a vague resemblance to their model. Here, using a large-scale phylogenetic comparative approach, we tested the hypothesis that inaccurate mimicry represents a transitional stage in an evolutionary trajectory toward accurate mimicry. We focused on two spider groups (Salticidae and Corinnidae) that both exhibit strong interspecific variation in the accuracy of mimicking ants (myrmecomorphy). We recorded the expression of nine morphological traits contributing to the myrmecomorphic habitus in 321 species and reconstructed their phylogeny based on UCE target enrichment sequencing. Our results suggest that ant mimicry evolved in some groups via gradual processes (rather than in jump-like events of strong morphological change) by the slow accumulation of mimicry-contributing traits. However, highly accurate mimicry states were highly unstable at the macro-evolutionary scale, with trends towards reversal, and the global selective optimum at low mimicry accuracy. Focussing on body constrictions, the most impactful modifications to the spider's body plan, we tested if the trend towards inaccurate mimicry can be explained with life-history trade-offs. Using two closely related jumping spiders – one ant-mimic with a prosomal constriction, and one non-mimic – we quantified the volumes of the central nervous system and the venom glands using micro-computed tomography. We found that the ant-mimic had a relatively smaller central nervous system and venom glands. These results, along with previous results on locomotory abilities and fecundity, suggest that ant-like body constrictions are under competing selection with life-history traits and may be strongly expressed only in highly selective environments.



Evolution of phalangeal morphology in an ecomorphological gecko radiation with incipiently expressed adhesive toepads

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Adhesive toepads, complex morphological structures enabling animals to exhibit astonishing climbing abilities, have evolved multiple times in geckos. Although well studied in a few exemplar clades in terms of function and morphology, their evolution is poorly understood, in part due to the scarcity of studies on incipiently developed toepad morphologies. One lineage within which incipient toepads has been suggested to have arisen is the genus *Cyrtodactylus* (bent-toed geckos), an ecologically diverse radiation, whose climbing members possess enlarged subdigital scales. The limited data available suggested that some members of the clade could be arranged in morphotypic series showing gradual changes in digit osteology from padless to pad bearing forms. With the phylogeny of the genus now much better resolved and knowledge about their microhabitat use greatly enhanced, we are now able to conduct a phylogenetically informed investigation of digit osteology using microCT data and applying 3D geometric morphometrics. We explored whether, and if so how, repeated gradual changes in digit osteology have occurred in scansorial species and whether such modifications differ depending on the locomotor substrate (e.g., arboreal vs. saxicolous species). We found that distinct morphological changes have arisen repeatedly in scansorial species descending from generalist ancestors. These adaptations are substrate specific for at least some of the phalanges, differing between arboreal and saxicolous species, although there were differential degrees of overlap in other phalanges.

Hardly Venus's servant— Morphological adaptations of *Veneriserva* to an endoparasitic lifestyle and its phylogenetic position within Dorvilleidae (Annelida)

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Endoparasitic annelids living inside another annelid host are known, particularly with regard to Oeonidae, but in general are poorly studied. The dorvilleid *Veneriserva pygoclava* is known from southern California, and its genus name (Latin = Venus's servant), alludes to the close association with the host aphroditid scaleworm *Aphrodita longipalpa*. Little is known on fundamental questions on the biology of *Veneriserva pygoclava*. What is the mode of reproduction? How do they feed? How do they penetrate the host? We have studied multiple parasitized hosts and *V. pygoclava* specimens, using an integrative approach, combining μ CT, histology and electron microscopy. 3D reconstructions from μ CT data of a parasitized *Aphrodita*, show the exact position of the parasites in their natural condition within the host's coelomic cavity. Ultrastructural investigations of the parasites revealed interesting adaptations to their life-style such as, the complete reduction of a gut, despite the presence of a functional jaw apparatus and a modified epidermis enabling nutrient uptake from the host's coelomic fluid. In addition to these, we also investigated spermatogenesis and oogenesis in *Veneriserva*. Sperm morphology indicates an external fertilization of eggs within the coelomic cavity of the host. Mature male and female parasites living inside the same mature host and the presence of juvenile *Veneriserva* within juveniles of *Aphrodita* suggest an obligate form of parasitism with a very early penetration of the hosts. In addition to our detailed morphological investigation, we have conducted a phylogenetic analysis showing the position of *Veneriserva* relative to other symbiotic/parasitic dorvilleids.



Functional morphology of the raptorial forelegs in Mantodea (Insecta)

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Prehensile raptorial forelegs are highly specialized prey capturing and grasping devices, best known among insects in praying mantises (Insecta: Mantodea). Mantodeans are exclusively predatory, showing strong morphological and behavioral adaptations towards the lifestyle of a generalist arthropod predator. However, there has been limited investigations concerning morphological variability of raptorial forelegs within mantodean species, particularly in relation to functional and comparative anatomy. Here, we used a comparative approach, focusing on the prevalent phenomenon of "female-biased sexual size dimorphism" (SSD) observed in almost every species of Mantodea. This SSD likely leads to a divergence in ecological niche exploitation between sexes, even by factors, such as prey size preferences. We examined the musculature of the raptorial forelegs in five different species (*Hierodula majuscula*, *Phyllocrania paradoxa*, *Pseudoxypops perpulchra*, *Hymenopus coronatus*, *Miomantis caffra*), exhibiting varying levels of SSD. We confirmed the presence of 15 extrinsic and 15 intrinsic muscles using μ CT and dissection, including one previously undescribed muscle present across all species, resulting in a comprehensive and detailed description of the musculature in Mantodea, accompanied by three-dimensional anatomical visualizations. Surprisingly, minimal observable differences were found in foreleg musculature, both between species and sexes. Additionally, we established homologies between the described muscles and the established nomenclature by Frieich and Beutel (2008), as well as discussed potential functional implications and homologies to the neuropteran *Mantispa styriaca* (Büsse et al., 2021) using the recently proposed leg muscle nomenclature by Aibekova et al. (2022). By elucidating the foreleg anatomy, particularly within the context of functionality and SSD, our findings contribute to the existing knowledge of insect raptorial forelegs. Furthermore, they foster our understanding of the underlying biomechanical system responsible for the predatory strike. Ultimately, this research paves the way for future comparisons with other insect taxa, facilitating a broader understanding of evolutionary adaptations in the context of insect predation.



Material composition and properties of cuticular components of centipede forcipules

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Many arthropods incorporate heavy metals into their piercing or biting structures, enhancing hard-ness, elasticity, and resistance to wear and tear. In the mouthparts of various arthropods, such as the mandibles, metals, like zinc, manganese, iron, and calcium can be found along the biting edges. Centi-pedes are renowned for their menacing appearance, due to their unique forcipules used for grasping, restraining, and piercing prey. These forcipules have evolved from locomotory legs and feature venom glands with an opening near their pointed tips for venom injection. Given their frequent exposure to high mechanical forces, we hypothesise that the distal cuticular components are reinforced. To inves-tigate these properties, we conducted energy-dispersive X-ray analyses on the cuticle of representa-tive species from all five centipede orders (Scutigermorpha, Craterostigmomorpha, Lithobiomorpha, Scolopenomorpha, and Geophilomorpha). Additionally, we examined cuticular sclerotisation gradi-ents using confocal laser-scanning microscopy based on autofluorescence signals of the cuticle mate-rials. To correlate the material composition of the forcipule with its mechanical properties, we ana-lysed breaking stress, breaking force, hardness and the Young's modulus of exemplarily taxa. Our find-ings reveal an increasing sclerotisation gradient towards the distal tarsungulum and an increased sclerotisation of the podomere joints across all studied taxa. Zinc incorporation was only observed in the tarsungulum of the forcipules in Craterostigmomorpha, Scolopenomorpha, and Geophilomorpha. However, the tarsungulum of species with incorporated zinc did not exhibit superior mechanical prop-erties compared to those without heavy elemental incorporation. Our results support the hypothesis that the forcipular tarsungulum in centipedes corresponds to the tarsus and the pretarsal claw, and that taxon-specific modifications of the forcipule may be correlated with environmental interactions.

Specific turbulence- and Chaoborus-induced morphs affect the streamlining properties of *Daphnia cucullata*

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Inducible defenses as a form of phenotypic plasticity are a wide-spread defensive mechanism in many species, especially in *Daphnia*. For example, *D. cucullata* is known to form different adaptive morphologies under changing environmental conditions. In this species, predator presence elicits defensive helmets. Defended animals perform better and survive more frequently in predation attempts. Another peculiarity of *D. cucullata* is that they develop these or at least similar defenses also in response to turbulence. The reason for this morphological alteration is so far unknown, but alterations of the head of this species are supposed to work as a multitool against different predators. Therefore, we investigated the three-dimensional morphology of the typical, the turbulence- and Chaoborus-exposed morph and analyzed their surface and volume alterations to identify potential costs. We furthermore measured the swimming velocities of these morphs. With the determined shapes and the measured velocities, we simulated the drag force under different angles of attack and determined the energy necessary to move through the water. In the light of previous findings and hypotheses on the so-called “Schwebefortsätze”, we also discuss biomechanical mechanisms that influence *D. cucullata*’s fitness under altered environmental conditions.



Morphology of fast-flowing stream tadpoles in the Afrobatrachian family Arthroleptidae

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The Arthroleptidae, a taxon of sub-Saharan frogs of the clade Afrobatrachia, include a number of remarkable taxa. Examples are frogs of the genus *Arthroleptis*, the only African direct developing anurans, or the charismatic Central African hairy frog (*Trichobatrachus robustus*). The majority of arthroleptid frogs inhabit forest habitats and the tadpoles of biphasic species often occur in flowing waters. Although Arthroleptidae account for almost twenty percent of all described African anurans, very little is known about most aspects of their reproductive biology. Especially the morphology of tadpoles and its relationship to their diverse lifestyles is poorly understood. To fill parts of this gap, we investigated the cranial morphology of four arthroleptid tadpoles – *Leptopelis parkeri* BARBOUR & LOVERIDGE, 1928, *Astylosternus occidentalis* PARKER, 1931, *Trichobatrachus robustus* BOULENGER, 1900 and *Nyctibates corrugatus* BOULENGER, 1904. All live in streams of lowland to montane forests, but the microhabitat of each species differs regarding water flow velocity. We found that the musculoskeletal systems of the tadpoles of *A. occidentalis*, *N. corrugatus* and *T. robustus* were highly modified compared to *L. parkeri*. Modifications included wide, robust and partly or completely fused cornua trabeculae, a fused and strongly chondrified *cartilago labialis* superior, as well as several modifications of the palatoquadrate. Some cranial muscles are highly hypertrophied accompanying the modifications of the robust chondrocranium. Beside these common modifications we observed also variation among the closely related *A. occidentalis*, *N. corrugatus* and *T. robustus*. The presence of a big processus hyoquadratis was only seen in the tadpole of *N. corrugatus*, indicating a possible autapomorphy of the genus *Nyctibates*. Collectively, our data revealed modifications of cranial morphology in arthroleptid tadpoles that include phylogenetic signals as well as adaptations to the different microhabitats they inhabit.

Molecular mapping of the neuroectoderm across phyla – conservation and divergence of brain regions between insects and vertebrates

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Gene expression has been employed for homologizing body regions across bilateria. The molecular comparison of vertebrate and fly brains has led to a number of disputed homology hypotheses. Data from the fly *Drosophila melanogaster* has recently been complemented by extensive data from the red flour beetle *Tribolium castaneum* with its more insect-typical development. In this review, we revisit the molecular mapping of the neuroectoderm of insects and vertebrates to reconsider homology hypotheses. We claim that the protocerebrum is non-segmental and homologous to the vertebrate fore- and midbrain. The boundary between antennal and ocular regions correspond to the vertebrate mid-hindbrain boundary while the deutocerebrum represents the anterior-most ganglion with serial homology to the trunk. The insect head placode shares common embryonic origin with the vertebrate adenohypophyseal placode. Intriguingly, vertebrate eyes develop from a different region compared to the insect compound eyes calling organ homology into question. Finally, we suggest a molecular re-definition of the classic concepts of archi- and prosocerebrum.



Mosaic evolution in the mushroom body lobes of a long-lived butterfly

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A species' adaptation to its environment often occurs through behavioural changes. Understanding how these changes take place in the brain is a central neurobiological question for which we use members of the Heliconiini tribe of neotropical butterflies.

The genus *Heliconius* actively feeds on pollen, a unique behaviour that, by exploiting a novel food source, lengthens these butterflies' life-span massively. Interestingly, pollen feeding with cognitive demands to locate sparsely distributed plants coincided with a massive expansion of the mushroom bodies. While we now know that this expansion has occurred through an up to seven-fold increase in Kenyon cells, it is largely unclear a) what information these additional Kenyon cells encode? and b) how the internal circuitry changed accordingly?

The focus of my project lies on the lobes, the anterior part the mushroom bodies where Kenyon cells, dopaminergic and mushroom body output neurons come together to form a learning circuit. This inherent complexity results in a collection of subneuropils where specific sub-populations of Kenyon cells connect to specific populations of the other two cell types. To answer how this internal circuitry changed along the massive increase in Kenyon cells, we stained brains of four Heliconiini species with several neurotransmitters and genetic markers, which allowed us to identify homologous lobe substructures in the lobes, which in contrast to *Drosophila*, are spheroid and subneuropils seem to be unified on first glance. By 3D segmenting 44 lobes of four species and two age groups, we were able to identify consistent expansions of the γ , α and β' lobes in *Heliconius* species, alongside differences in age groups and inside clades. Neurotransmitter stainings, tracer injections and developmental data further indicate that in *Heliconius* species the mushroom bodies have evolved to accommodate behavioural innovation through a mosaic change of Kenyon cell populations.

Development of Johnston's organ in the antennal nervous system of the locust

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Johnston's organ (JO) is a multisensory organ in the insect antenna mediating audition, flight control, gravity sensing, and other behaviors. In the hemimetabolous locust, such sensory systems must be functional upon hatching and so develop during embryogenesis. The locust antenna is a proven model system for studying sense organ development and we have used a spectrum of epithelial, mitosis and neuronal markers to investigate how neurogenesis and axogenesis generate and wire up the sensory neurons of JO. The first sense organ precursor (SOP) of JO appears within the epithelial domain of the pedicel at a third of embryogenesis, followed rapidly by further precursors each generating a clone of progeny. The location of these clones is stereotypic across individuals and ages suggesting there is an underlying epithelial coordinate system that regulates JO topography. Clone numbers and lineage size increase in discrete steps so that at mid-embryogenesis the cellular organization of JO resembles that of the adult. During axogenesis, growth cones from cell clusters of JO fasciculate selectively with pre-existing pioneer tracts according to their location in the epithelium resulting in a topographic projection pattern. Pioneer and JO axons also express the cell surface lipocalin Lazarillo which regulates axogenesis in the antennal nervous system. The axonal projections from JO terminate near protocerebral tracts bearing the processes of identified premotor interneurons that regulate flight behavior. Immunolabeling against the vesicular acetylcholine transporter (vAChT), which packages acetylcholine into vesicles, and choline acetyltransferase (ChAT), which synthesizes acetylcholine, failed to detect vAChT at 70% of embryogenesis, but vAChT was present in olfactory and mechanosensitive sensilla at 90% and 99% of embryogenesis. In first instar nymphs, vAChT was present in the axons of olfactory sensilla, and ChAT in their denites and somata. These data are consistent with a functional cholinergic system in the antenna on hatching.

Dyadic Attraction and Allocentric Vectorial Integration in Density-Dependent Desert Locust Marching

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Gregarious juvenile locusts traverse kilometers relentlessly across expansive terrains. The sheer scale of locust collectives and the remoteness of plague-impacted sites, particularly in Africa, have historically impeded thorough understanding and systematic control of locust plagues. Our study employs a two-pronged approach: integrating field observations from the 2019 locust outbreak in Kenya with a groundbreaking custom-developed 360-degree immersive Virtual Reality (VR) system. A focal locust is immersed in a virtual marching band of lifelike 3D locust models in this VR environment. This innovative method allows for unparalleled experimental control, enabling in-depth analysis of individual and collective behaviors, which was previously unfeasible for this species.

Our findings reveal that the focal locusts can align and march with groups of lower density, provided that these groups are cohesive. Even in cohesive groups, multiple forces can exert on the individual, and we discovered that regressive visual cues in isolation could elicit sustained attraction in the focal locust. This finding suggests that attractive pull forces serve as the fundamental driving mechanism in forming marching bands and that these forces can act over distances, facilitating the joining of individuals to established bands. This attractive force operates through dyadic interactions. A single, sustained virtual locust movement can also induce alignment in the follower locust. Once deconstructed into dyadic interactions, collective locust marching can be conceptualized as allocentric vector integration predicated on the relative spatial positioning of other locusts.

This study illuminates the mechanisms of density-dependent locust marching, offering a foundation for novel lines of inquiry and innovative intervention strategies. Notably, the employment of VR technology, which necessitates only one desert locust instead of hundreds, sets the stage for future in-depth analyses by facilitating integration with brain-wide imaging and molecular techniques. This research represents a milestone in the realm of locust collective behavior studies.



Do you smell what I smell? Social modulation of olfactory processing in locusts

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Plagues of the desert locust, *Schistocerca gregaria*, pose a severe threat to the food security of millions. Central to their swarm formation is a crowding-induced phase transition leading to large fluctuations in population density. Such drastic changes are postulated to profoundly impact how locusts process environmental and social information during their devastating foraging campaigns. In this study, we investigate the associated sensory adaptations with how locusts sense and select feeding sites. We first employed a Bayesian decision-making rule for integrating different information classes and revealed olfaction to play a critical role. Therefore, we continued to investigate the implementation of socially induced changes in the olfactory system. The locust antennal lobe – the first processing center for olfaction – has a highly distributed structure with over a thousand microglomeruli, making the investigation of odor coding challenging. We developed an in vivo functional calcium imaging protocol for recording the activity of antennal lobe projection neurons. This allowed us to map the combinatorial olfactory representation of food odor cues both under a simulated social context and alone. Our results demonstrate an increase in the overall activity of gregarious animals' projection neurons to food odorants in the presence of conspecific odor, suggesting an adaptive mechanism to facilitate group foraging. We further corroborate these results with behavioral assays, investigating the role of socio-visual/olfactory and nutri-visual/olfactory cues during patch choice. Here, gregarious locusts were strongly attracted to the combination of social and food cues. In contrast, a social modulation of neither olfactory processing nor patch preference occurred in solitary animals. Locusts face unpredictable environments and rapidly changing social structures, rendering them an ideal model system for studying social plasticity. Our study provides insights into their phase-dependent sensory ecology and sheds light on the requirements underlying the transition between the solitary and gregarious phase.

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

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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 and reduced protein abundance with immunofluorescence. This study provides a first functional proof of both chemo co-receptors being involved in predator perception and probably chemo perception per se.

Discovery and functional characterization of RYamide signaling in *Rhyparobia maderae*

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Transplantation studies located the circadian clock of the Madera cockroach *Rhyparobia (Leucophaea) maderae* to the accessory medulla (AME), ventromedially to the medulla of the brain's optic lobes. The AME receives photic entrainment from compound eye photoreceptors and orchestrates circadian rhythms in physiology and behavior synchronized to the external 24h light-dark cycles. About 240 neurons innervate the AME which are abundant of partly co-localized neuropeptides. The best studied neuropeptide of the insect circadian clock is pigment-dispersing factor that controls rest-activity cycles. However, the identity and functions of most other neuropeptides of the circadian clock are not known. In this study, we identified three novel neuropeptides which are encoded on the ry-amide gene in *R. maderae* whose precise expression and function have not yet been determined. We identified the ry-amide gene by transcriptome analysis of the CNS and predicted three RYamides: RYa-1 (pQQFYPPGGRY-NH₂), RYa-2 (GSSTFWSGSRY-NH₂), and RYa-3 (NDRFFIGSRY-NH₂). By applying direct tissue profiling using MALDI-TOF mass spectrometry (MS) and Orbitrap MS of the AME, we obtained all predicted RYamides. Using a polyclonal antiserum which recognizes the C-terminal sequence RY, we described its spatial distribution of RYamide in the clock. For functional analysis within the clock system, we applied quantitative immunocytochemistry on whole mount brains and Ca²⁺ imaging experiments using synthetic RY-1 and RY-2 on primary AME cell cultures at different Zeitgeber times. Results show that RYamides are highly expressed in the AME at the beginning of the day (ZT1) while on mid-day (ZT6) and early night (ZT12) expression levels are low. Previous studies of RYamide in *Drosophila melanogaster* revealed RYamide expression in the gut neurons, suggesting that RYamides might regulate cyclical feeding. Therefore, quantitative immunocytochemistry and qPCR experiments of the peripheral nervous system of satiated or food-deprived cockroaches were performed to investigate transcription levels and peptide levels of RYamides.

Dynamic Contrast 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 environment with particularly high dimensionality. Natural visual scenes contain different spatial frequencies, which can vary across parts of the visual field (Bigge et al., 2021, *Curr. Biol.* 31, 6: R280-R281), 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. One such example can be found in hawkmoths, for which it was shown that motion neurons in the lobula complex are sensitive to contrasts over a large range of light intensities (Stöckl et al. (2017). *Proc R Soc B* 284: 20170880). These properties can only be explained by dynamic processing of the visual input. An important neuropil for such is the lamina, the first processing stage in the insect visual system. Its main relay neurons, the lamina monopolar cells (LMCs), receive information directly from the photoreceptors and play an important role in shaping contrast, luminance, spatial and temporal information. To investigate the role of LMCs in visual processing in hawkmoths, we reconstructed the anatomical fine structure of the lamina in 2 hawkmoth species, which led us to propose a new classification of LMC types in this insect group. Using intracellular recordings, we investigate the physiological properties of different LMC types. We demonstrate that, similar to the fruitfly, L1 and L2 types encode contrast rather than luminance. Combining the physiology and anatomy of these neurons allows us to identify their role in the motion vision pathway, thereby explain the dynamic properties' of the hawkmoths' motion responses. Further, we can establish potential homologies and differences in LMC anatomy and function across insect groups, which ultimately provides a better understanding of how this neuropil contributes to dynamic processing in the insect visual system.

Midsession reversal learning performance in harbor seals (*Phoca vitulina*)

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Cognitive flexibility is the ability to adapt behavioral responses to unexpected environmental changes. A common paradigm to measure cognitive flexibility is reversal learning (RL). In a RL task, the subject first learns that choosing the positive stimulus is rewarded and choosing the negative stimulus is not. After the acquisition of the task, the reward contingencies change and the previously positive stimulus is no longer associated with a reward, but the previously negative stimulus is. While in previous RL studies, the harbor seal's performances in a visual and spatial RL experiment was assessed, the aim of this study was to analyze the performance of harbor seals in a variant of the RL paradigm, the midsession RL. In a midsession RL task, the reversal occurs in the middle of the session, and mostly time since the start of the session or a "win/stay-loose/shift" strategy is underlying the responses of the experimental subjects. Two harbor seals participated in our spatial midsession RL experiment, Seal 1 had RL experience and seal 2 was naïve with respect to RL experiments. Seal 1 learned the midsession reversal task very quickly and its response behavior was in line with a "win/stay-loose/shift" strategy. Seal 2 needed more experimental sessions to learn the task and showed anticipatory and perseverative errors indicating that seal 2 had used the passage of time from the start of the session to the reversal point as a cue to reverse. Thus, harbor seals use different strategies to learn a midsession reversal, presumably depending on their previous experience with similar tasks. Whereas strategies have already been shown to underly harbor seal behavior, this study provides evidence that the seal's recently described sense of time can control behavior.

Age-related differences in the sleep of juvenile and adult zebra finches

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Despite their phylogenetic differences and distinct pallial structures, mammals and birds show similar electroencephalography (EEG) traces during sleep, consisting of distinct rapid eye movement (REM) sleep and slow wave sleep (SWS) stages. Studies in human and a limited number of other mammalian species shows that this organization of sleep into interleaving stages undergoes radical changes during lifetime. Do these age-dependent variations in sleep patterns also occur in the avian brain? Does vocal learning have an effect on sleep patterns in birds? To answer this question, we recorded multi-channel sleep EEG from juveniles and adult zebra finches for several nights. Whereas adults spent more time in SWS and REM sleep, juveniles spent more time in intermediate sleep (IS). The amount of IS sleep was significantly larger in male juveniles engaged in vocal learning compared to female juveniles, which suggest that IS sleep could be important for learning. In addition, we observed that the functional connectivity increased rapidly during maturation of young juveniles, and was stable or declined at older ages. Synchronous activity during sleep was larger for recording sites in the left hemisphere for both juveniles and adults, and generally intra-hemispheric synchrony was larger than inter-hemispheric synchrony during sleep. A graph theory analysis revealed that in adults, highly correlated EEG activity tends to be distributed across fewer networks that are spread across a wider area of the brain, whereas in juveniles, highly correlated EEG activity is distributed across more numerous, albeit smaller, networks in the brain. Overall, our results reveal significant changes in the neural signatures of sleep during maturation in an avian brain.



Impact of the peripheral fat body clock on lipid metabolism and health in *Drosophila*

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The body clock of animals consists of a central circadian pacemaker in the brain and circadian clocks in peripheral organs. In humans, desynchronisation of these clocks due to modern lifestyles can lead to obesity or metabolic diseases, yet the impact of circadian clocks on lipid metabolism is poorly understood. We previously reported that a body-wide loss of endogenous clocks in period mutant fruit flies results in altered intermediary lipid metabolism and a loss of rhythmic lipid transport in the hemolymph^{1,2}. Now we started to specifically address the role of the peripheral clock in the fat body, a central tissue in lipid metabolism. We specifically impaired the fat body clock in female flies and performed lipidomic and behavioral analyses. As expected, loss of the fat body clock did not affect the rhythmicity of locomotor activity. On normal feed, flies with a defective fat body clock had a significantly reduced lifespan compared to controls. Starvation resistance or total triacylglycerol levels were similar in bodies of control and dysfunctional fat body clock mutant flies. However, LC-MS analysis revealed age- and lipid species-specific differences in triacylglycerols. Interestingly, propionyl-, butanoyl- and malonyl-carnitine levels were significantly higher in the heads and bodies of clock-deficient flies compared to controls, whereas the long-chain acylcarnitine pool appears to be unaltered. These results suggest that the peripheral clock in the fat body is involved in the proper timing of the mitochondrial CoA pool.

¹Schäbler et al. (2020) Cell Mol Life Sci 77:4939

²Amatobi et al. (2023) J Lipid Res, in press.



Tracheal terminal cells in *Drosophila* show a limited immune response to avoid cell death

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The *Drosophila* tracheal system, with dorsal trunks and primary, secondary and terminal branches, shares many structural and functional similarities with the mammalian lung. To protect the large surface area from invading and colonising pathogenic bacteria, the trachea is equipped with a sophisticated innate immune system. By overexpressing the pattern recognition receptors PGRP-LC and PGRP-LE throughout the tracheal system, activation of the Imd pathway, the major branch of the epithelial innate immune system, induces a strong immune response. This immune response includes the expression of antimicrobial peptides in all tracheal cells, but appears to exclude the so-called terminal cells. The terminal tracheal cells (TTCs) are responsible for gas exchange, the most important function of the respiratory organs. TTCs are closely connected to all tissues and organs to ensure oxygen supply. Similar to endothelial tip cells, these cells exhibit structural plasticity in response to nutrient cues and hypoxia. When PGRP-LC is overexpressed in these cells, there is a marked reduction in branching, cell damage and ultimately cell death. In addition, the larvae showed a significantly increased sensitivity to hypoxia. Further analysis revealed that the induction of cell death is achieved by a branch of the IMD pathway at the level of TAK1, which links immune activation to JNK signalling, which in turn activates the cell death machinery. These highly sensitive cells may undergo a limited immune response in order to avoid cell death due to the absence of PGRP-LC in the terminal cell.



A mitochondrial sirtuin shapes the intestinal microbiota by controlling lysozyme expression

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Sirtuins (Sirts) are evolutionarily conserved NAD⁺-dependent protein deacetylases, which act as cellular sensors to adapt the properties of cells and organs to cope with environmental changes. In the gut of the fruit fly *Drosophila melanogaster*, we observed that dSirt4 is the only sirtuin that is strongly up-regulated in response to a nutritional stressor, a protein-depleted diet. Using proteomic analyses and activity measurements, we were able to show that dSirt4-deficient flies exhibit a massively increased expression and activity of different lysozymes in the intestine. This effect was also observed in flies with silencing of dSirt4 exclusively in the enterocytes of the intestine. Interestingly, the strongly increased lysozyme expression did not alter the total bacterial load in the intestine, but changed the composition of the microbiota by reducing the number of gram-positive bacteria, resulting in a shift towards gram-negative Acetobacteraceae. Since this effect was not observed in a lysozyme-deficient background, the changes in the microbial composition can be attributed to the dSirt4-dependent lysozyme expression. Additionally, the deficiency of dSirt4 in enterocytes reduced lifespan of flies, which was also observed in flies ectopically overexpressing lysozyme in enterocytes. This implies that strong lysozyme expression leads to a dysbiotic state, which finally leads to premature death of the animals.



Comparative structural insights into insect and fungal chitin synthases and their inhibition by conventional pesticides

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Chitin, a polymer of $\beta(1,4)$ -linked N-acetylglucosamines, is an essential component of insect extracellular matrices such as cuticles and peritrophic matrices. While the cuticle serves as an exoskeleton, which also protects from desiccation and microbial infection, the peritrophic matrix in the midgut enhances digestion and provides antimicrobial defense. Targeting chitin biosynthesis as a pest management strategy in agriculture has therefore gained attention due to its critical role in insect growth and development. Several commercial insecticides have been shown to disrupt chitin biosynthesis, including the benzoylphenyl urea (BPU) compound diflubenzuron (DFB), which leads to structural changes of the cuticle and appears to bind and inhibit the chitin synthase. However, DFB's mode of action in inhibiting insect chitin synthase and the mechanisms underlying resistance to this compound are poorly understood. This knowledge gap poses a challenge for the safe and sustainable use of BPUs. Our study aims to investigate the structural basis of chitin synthase inhibition by DFB. Using bioinformatic approaches based on recent cryo-EM structures of fungal chitin synthases, we performed comparative structural analyses of chitin synthases from different sources that are either susceptible or resistant to DFB. Specifically, we examined the three-dimensional structures to identify potential binding sites by molecular docking algorithms and elucidate the mode of inhibition.



Zooplankton is challenged by cyanobacteria: decline of energy reserves in *Daphnia magna*

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Eutrophication and rising temperatures in lentic ecosystems increase the danger of algal blooms. Phytoplankton is often dominated by cyanobacteria, which reduce the grazing pressure by impeding the growth of their consumers via toxins. As water fleas are unselective filter feeders, the presence of cyanobacteria and their uptake prevent adequate digestion in the intestinal system of daphnids by inhibition of proteolytic enzymes. Especially at elevated temperatures, a shortage of substrates for energy metabolism can be the result, impairing growth and reproduction.

Daphnia magna was exposed to different combination of green algae (*Desmodesmus subspicatus*) and cyanobacteria (*Microcystis aeruginosa*), varying the available food in quantity and quality. The *Microcystis* strains used (PCC 7806, NIVA Cya 43) were free of microcystin, but produced inhibitors of trypsin or chymotrypsin, respectively. After incubation for 48 hours at 10°C, 20°C or 30°C, *Daphnia* specimens were analysed concerning their content of carbohydrates, lipids and proteins. Exposition of daphnids to cyanobacteria-containing food resulted in a decline of energy reserves, which was aggravated by rising temperatures. The impacts on the nutritional state were comparable to the magnitude of effects in response to starvation.



Effects of warming on the metabolic disruption caused by a hypolipidemic drug gemfibrozil in a model marine bivalve *Mytilus edulis*

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Hypolipidemic drugs are ubiquitous in global markets, and their incomplete removal from wastewater treatment plants often results in their release into aquatic environments, posing a risk of toxicity to resident biota. We propose that these drugs can act as metabolic disruptors in non-target marine organisms, such as the mussel *Mytilus edulis*, which accumulate pollutants through filter feeding, and that these effects may be exacerbated by warming. To investigate this hypothesis, we studied the mechanisms of toxicity of the commonly used lipid-lowering ug, gemfibrozil (GFB), on the cellular metabolism and stress-responsive systems of *M. edulis*. Mussels were exposed to 25 µg L⁻¹ GFB for two weeks at control (15°C) and elevated (20°C) temperatures. Our results showed that GFB exposure caused significant oxidative stress in *M. edulis*, as evidenced by the accumulation of lipid and protein oxidation products, suppression of total antioxidant capacity, and depletion of glutathione. Additionally, exposure to GFB resulted in altered energy metabolism, including elevated lipid:carbohyate ratios and a decrease in tissue cholesterol and triglyceride levels. Acyl-CoA oxidase activity, a key lipid breakdown enzyme, was also elevated in GFB-exposed mussels, particularly when combined with elevated temperature. Furthermore, GFB exposure led to the accumulation of methylglyoxal and activation of the glyoxalase system, indicating dicarbonyl stress.

Overall, our findings suggest that GFB disrupts normal lipid and carbohyate metabolism and causes oxidative stress in non-target marine organisms such as *M. edulis*. This could have adverse implications for the health of coastal mussel populations. Interestingly, elevated temperature did not enhance the toxicity of GFB. Moreover, GFB had no effect on amino acid metabolism or urea cycle, irrespective of temperature. Our results emphasize the need for a better understanding of the effects of environmental pollutants on non-target organisms and highlight the potential risks associated with the release of pharmaceuticals into aquatic ecosystems.

Osmotolerance reflected in mitochondrial respiration of Blue Mussel populations from three different habitat salinities

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Mussels from the *Mytilus edulis* species complex experience a salinity gradient from the North Sea into the Baltic Proper ranging from 32 psu to 5-6 psu. As osmoconformers, mussels adjust their internal osmolarity to match that of their surroundings. This adaptive strategy poses a significant challenge to the metabolic machinery of the mussels, including their mitochondria. We hypothesised that the osmotic optima for the mitochondrial function of mussels matches the prevailing habitat salinity. To test this hypothesis, mussels from three populations along the salinity gradient were assessed: north Baltic Sea (*M. trossulus* zone), south Baltic Sea (transition zone) and North Sea (*M. edulis* zone). The mitochondrial performance curve was generated by measuring respiration of isolated hepatopancreas mitochondria in the resting (LEAK) and actively phosphorylating (OXPHOS) states (indicative of the futile proton leak and ATP synthesis capacity, respectively) at a range of osmotic concentrations. The habitat salinity shift from the north to the south Baltic Sea was reflected in a shifted peak performance of the mitochondria towards a higher osmotic concentration. Mitochondria from North Sea *Mytilus* exhibited a better performance at higher osmotic concentrations and showed a wider tolerance range than their Baltic Sea congeners. The osmotic concentration also affected mitochondrial coupling efficiency, reserve electron transport system capacity and reserve cytochrome c capacity. Baltic Sea populations appear to have traded off an adaptation to low salinities for a narrower mitochondrial tolerance range resulting in a more specialized mitochondrial phenotype, while North Sea populations have mitochondria with a more general functioning phenotype.



Establishment of an Omics based biomonitoring pipeline: Fishes in the Elbe estuary

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Holistic transcriptome studies provide a mechanistic understanding of an organism's response to changing environmental conditions. Recent studies have illustrated the importance of considering multi-stress-environments in evaluating the response of an organism, with sometimes opposite or accumulated responses compared to single-stressors. Here we link gene expression patterns in wild fish within their natural habitat with spatiotemporal gradients in the tidal Elbe estuary. Tissue specific transcriptomic adjustments are considered incorporating liver as key metabolic organ and gill with respiratory, excretory and immunological functions. In parallel, we study the bacterial composition in the gills, as the function of this tissue requires high connectivity between surrounding water and the animal's bloodstream, making it an important entry point for pathogens.

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, we caught fish with a stow-net vessel along the main channel of the tidal Elbe between spring 2021 and summer 2022. RNAseq, 16SrRNA metabarcoding, shotgun metagenomics and LC-MS3 steroid hormone determination are used in this study. Clusters of co-expressed genes and co-occurring bacteria were identified and correlated with physiological and abiotic metrics. The two-factorial design allows individual genes and pathways to be tracked over time and space, indicating for example responses to xenobiotics near cities whereas oxygen depletion and heat in summer months elicit strong immune responses. Distribution, function and possible pathogenicity of bacterial taxa are now inferred by metagenome analyses. Population structure and possible local adaptations are investigated using population genetic tools on the transcriptomic datasets. Finally, a cause-and-effect modeling approach is meant provide insights into the effects of abiotic factors and bacterial pathogens through the activation of specific gene cascades on physiological measures of fish health.



The muscle transcriptome of non-healthy harbour porpoises (*Phocoena phocoena*) hints at oxidative stress, but not hypoxia

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The harbour porpoise (*Phocoena phocoena*) in the North and Baltic Seas is impacted by increasing anthropogenic pressures such as shipping, noise and pollution. Exceeding contaminant exposure can impair the immune system and growing pathological lesions in the respiratory tract, mainly caused by parasites or bacteria, have been found in harbour porpoises. Further, they have been observed to not use their complete lung volume. Whether this causes lower oxygen uptake, impaired diving ability and ultimately reduced foraging success, has not been studied. This project aims to analyse whether harbour porpoises developed molecular adaptations to compensate reduced oxygen supply, thus remaining viable and competitive despite high parasitic load.

To analyse molecular adaptations to reduced oxygen levels, we generated the first muscle transcriptomes of the harbour porpoise and performed comparative transcriptome RNA sequencing. We compared muscle transcriptomes of harbour porpoises suffering from bronchopneumonia and severe lesions in the respiratory tract with muscle transcriptomes of healthy porpoises.

Our results indicate that harbour porpoises do not suffer from hypoxic conditions in the muscles exacerbated by pathological lesions in the lung, but may experience elevated oxidative stress. Higher antioxidant gene expression in the muscles of non-healthy harbour porpoises might function as a compensatory effect to high reactive oxygen species production and accumulation in the muscles. Regeneration and selective proteasomal degradation were found simultaneously upregulated, suggesting an adaptation in a fast and finely tuned switch. Transcripts and processes involved in anaerobic and lipid metabolism were found elevated in non-healthy harbour porpoises which may fuel additional energy supply for energy-consuming processes such as regeneration and translation in the high energy-consuming muscles.

These findings hint at a defined muscle response to oxidative stress which may be caused by severe pathological lesions in the respiratory tract and represents an important step to understand the molecular adaptations to stress in cetaceans.



TimeTeller: A novel tool to investigate molecular circadian clock function in individuals

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Circadian clocks are biological time-keepers that have evolved in virtually all living organisms, including all animals, as an adaption to the predictable geophysical day. These oscillators are recognised to significantly modulate a multitude of key cellular and physiological processes as well as behaviour. Determining clock phase and/or proper clock function in an individual usually necessitates longitudinal data of a behavioural or physiological measure, for example, general activity or blood pressure rhythms, respectively. However, collecting information about the state of the underlying molecular oscillator, i.e., clock gene or protein expression, to infer the clock status in a given tissue or individual animal is usually mostly not feasible, and time-series sampling in a population must be used instead, especially in genetically or phenotypically heterogenous and non-model species as well as in the field this is difficult or impossible to date. Therefore, there is a need for tools to measure the functional state of the molecular circadian clock. Importantly, the clock mechanism, i.e., core clock genes, are well described in many species, although there are few tools that capitalise on this knowledge of the clock as a multi-dimensional stochastic oscillator and analyse it as a system. We propose a novel machine learning tool, TimeTeller (TT), that estimates circadian clock phase with great accuracy and function from expression of about a dozen clock genes by modelling the multi-dimensional state of the clock. We demonstrate TT's potential to investigate circadian clocks by applying it to rodent and primate microarray and RNA-seq data. Examples for visualising and quantifying the global structure of the clock will be given, which also allow stratifying individual transcriptomes and globally compare clocks across individuals, conditions, and tissues. This highlights TT's potential for studying circadian clock function in individuals by overcoming previous limitations for sampling time-series data.

Unraveling the biting past of the "non-biting midges"

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Chironomidae is a species rich group of dipterans, informally called non-biting midges. As the name implies, most extant species appear to forego feeding during their adult stage, utilizing it solely for reproductive purposes. Adult mouthparts are reduced and lack biting structures such as sclerotized mandibles and laciniae, present in other related groups, like the biting-midges (Ceratopogonidae). Nevertheless, notable exceptions exist within the extant fauna. Species of the genera *Archaeochlus* and *Austrochlus*, from southern Africa and Australia, respectively, have toothed sclerotized mandibles. Another closely related genus from the Afrotropical region, *Afrochlus*, displays poorly sclerotized smooth mandibles. Interestingly, our research has unveiled several species with functional biting mouthparts from fossil records. The diverse morphology of head appendages in these extinct groups implies a wide range of feeding habits. The presence of functional mouthparts is scattered across the chironomid phylogeny indicating a complex evolutionary history involving multiple instances of gains and/or losses. This study aims to report new species of biting Chironomidae from the fossil record using light microscopy and micro-CT scans of amber inclusions. The detailed morphological description of the extant and extinct mouthparts can elucidate potential feeding habits and phylogenetic relationship with other chironomids. Our results suggest independent events of loss and reacquisition of biting mouthparts, possibly linked to the diversification of feeding habits. Additionally, we present compelling evidence of mammalian hosts, inferred from the presence of red blood cells surrounding the head and body of fossil Chironomidae.



Phylosymbiosis of fungus-gardening ant symbiomes

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Phylosymbiosis, which is concordance between the phylogenetic history of a microbiome and the phylogenetic history of the host is a concept that may be used to examine whether hosts and microbiomes co-evolve or are the product of environmental acquisition. The “neoattini” fungus-gardening ant symbiosis offers a unique opportunity to investigate the connection between phylosymbiosis and coevolution, as it contains multiple symbiotic partners, some (e.g., the host ants and fungal symbiont) are known to have a long coevolutionary history. Here we apply 16S rRNA gene analysis in combination with analysis of nuclear genes and SNPs (single nucleotide polymorphisms) to examine for phylosymbiotic patterns between host ants, fungal symbionts, and bacterial microbiomes of five species of *Trachymyrmex* found in southern North America. As our study extended across a region spanning >1000 km we also examined for geographic components of population structure. While there were some minor geographic components, we report strong coevolutionary signals between host ants, fungal symbionts and bacterial microbiomes. These results demonstrate that the fungus gardening ants represent a community composed of host ants, fungal symbionts and bacterial taxa that retain structural coherence throughout ecological and evolutionary scales.

Sensitivity to mimicked neonicotinoid exposure varies across species and sex of non-target plant bugs (Heteroptera: Miridae)

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It is increasingly recognized that the terrestrial environment is widely contaminated with various pesticides, and insecticides in particular are considered to be a major driver of the insect decline. Despite the enormous diversity of insects, only a handful of indicator species are tested in standardized laboratory and (semi-) field studies prior to insecticide registration. An important but understudied issue is the response of whole insect communities and potential differences in the susceptibility of non-target insects. Here, we used the previously neglected group of plant bugs (Heteroptera: Miridae) as a model to study the effects of insecticide exposure on non-target insect communities. Plant bugs are abundant in field margins and open grasslands and thus represent a group of non-target insects likely to be exposed to spray drift. We conducted field and laboratory experiments to assess the effect of insecticide contamination with the semi-systemic neonicotinoid Mospilan®SG on plant bug communities and to compare the response of different species. Mimicking exposure at 1 m field distance, we found an overall reduction of plant bugs by 78%. The different population dynamics of individual species observed in the field were partly reflected in the susceptibility of species to topical insecticide exposure and to feeding on insecticide-treated plants in the laboratory. Remarkably, males of *Leptopterna dolabrata* were significantly more sensitive to insecticide treatment than females. Our results suggest that pesticide registration requirements may significantly underestimate the effect of insecticide exposure on non-target insects. In addition, the limited number of standard model organisms may not be sufficient to fully understand the environmental impact of insecticides and their potential effects at the community level.



Improving the phylogenetic tree of extant hominoid species by character weighting of amino-acid-substitutions and attempts to deduce the phylogeny of well-preserved fossil human skulls

Udo Rempe

Uni Kiel

For the reconstruction of phylogenetic trees from protein sequences it is necessary to support the reconstruction to differences at positions for which it is probable that there occurred only one change during the evolution of the systematic group considered (A. REMANE). For an evolutionary tree of the extant hominoids the 13 mt-DNA encoded proteins are used. Differences of positions where more than two amino acids occur within or between species are left away. From the remaining dimorphic positions those with both amino acids within one of the species are to too be excluded. Such the phylogenetic relationships of the Hylobatidae can now largely be deduced. Moreover, the proteins show that *Homo sapiens denisova* switched off first and *Homo sapiens neanderthalensis* later from humans like today *Homo sapiens*.

From 17 well preserved fossil upper human skulls phylogenetic relationships are attempted to be estimated. Changes caused by directional selection were attempted to eliminate.

After *Ardipithecus ramidus* switched off, we get a first group with *Australopithecus transvalensis* Sts5, the Dmanisi skull 5, a first right missing link and a first left missing link. From the first right missing link switch off: KNM ER 1813, KNM ER 3733, *Homo naledi* and to-day *Homo sapiens*. From the skull shape of to-day humans developed *Skhul V* and *Homo sapiens neanderthalensis*. From the neanderthals switch off *Homo sapiens rhodesiensis* and *Homo sapiens petralona*. From the first left link switch off *Australopithecus habilis* OH2, *Australopithecus floresiensis*, *Australopithecus afarensis* A.L. 444.2, *Australopithecus rudolfensis* and the second left missing link. From the second left missing link originate *Australopithecus robustus* DNH7 and *Australopithecus boisei* OH5.



POSTER ABSTRACTS

List of posters can be found [here](#)

Muted males - assessing the importance of vibrational communication in *Pisaura mirabilis* courtship

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Vibratory communication is common amongst many different taxa - although seldom referenced - and has been described for several species of spiders. Spiders make use of vibrational cues or signals for varying purposes, ranging from hunting to species recognition and reproduction. In a reproductive context, vibrations can suppress aggression and convey information about the condition of the signalling individual.

The Nursery Web Spider *Pisaura mirabilis* is known for its nuptial gift giving behaviour - males wrap prey in silk and present it to females before copulating. Additionally *P. mirabilis* males display vibrational courtship by tremulating their opisthosoma and transferring the produced vibration onto the substrate via their legs. This vibrational courtship display appears to be condition-dependent and can be interpreted as an honest signal. While different modalities of this behaviour are already being discussed, the precise role of the characteristic vibration in *P. mirabilis* courtship is still uncertain.

In this study I assessed the role of courtship vibrations and their impact on courtship behaviour as well as copulatory success in *P. mirabilis*. To this end, I manipulated male spiders by placing a drop of melted paraffin wax between prosoma and opisthosoma, impairing their natural tremulating behaviour. Qualitative analysis and comparisons of their vibrations showed some difference between muted and control males. Subsequently, mating trials were conducted observing either muted- or control-males with standardized females, documenting courtship behaviour and copulatory success. I found that females were less likely to mate with a muted male in comparison to control males, while both offered a nuptial gift. This finding can aid us in further determining the role of biotremology in the multimodal communication context of *P. mirabilis* courtship.

Infanticide in burying beetles: Unveiling the dark side of parental care

Lena Zywucki, Seher Kaya, Sandra Steiger

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Infanticide is a widespread phenomenon in the animal kingdom, often occurring in the context of parental care. While infanticide is commonly committed by non-related competitors, certain taxa, such as the genus *Nicrophorus*, parents kill their own larvae to regulate brood size. However, our understanding of the factors that influence the extent of parental infanticide remains limited. In this study, we use three burying beetle species that differ strongly in their dependence on parental care, from facultative to obligate. We investigated whether the regulation of brood size varies across the species and whether factors such as resource availability, sex or the number of parents caring for the brood influence the degree of infanticide. Our findings revealed that both resource availability and species significantly influence infanticide, as does the level of parental care provided to the offspring. Additionally, we observed differences in brood size regulation between uniparental males and biparental parents in the most dependent species. For the independent species, we further examined the effect of parental presence on larval survival and found that a higher number of larvae survived when no care was provided. The latter result confirms the occurrence of infanticide in the context of parental care. By investigating brood size regulation in several species and considering various factors, including resource availability and parental care, we provide valuable insights into the complex dynamics of infanticide and its evolutionary implications.

Emerging from Solitude: Understanding Behavioural Transitions of Desert Locusts through Visual Stimuli in Solitarious and Gregarious Phases

Nina Schwarz, Laura Schröder, Chi-Yu Lee, Sercan Sayin, Einat Couzin-Fuchs

Universität Konstanz

Desert locusts undergo a drastic phase change, transitioning between a solitarious and gregarious form that can lead to a devastating swarming behaviour that threatens agricultural food supply. Upon an increase in population density solitarious individuals of the desert locust, *Schistocerca gregaria* undergo a rapid gregarization, leading to alterations in behaviour and preferences. While much research has focused on the behaviour of gregarious locusts, the behaviour of solitarious individuals and the transition into the gregarious phase are less understood. Our study investigates responses of solitarious and gregarious locusts to visual motion stimuli: wide-field optic flow (motion of black dots) and movement of virtual and real conspecifics tested in a preference arena, a trackball and virtual reality (VR) setups.

We first noted no significant differences in optomotor responses of solitarious and gregarious locusts, suggesting similar visual processing capabilities. In the VR environment, a moving band of virtual animals served as a stimulus, revealing distinct behavioural patterns. Gregarious locusts exhibited enhanced alignment and directional movement within the virtual swarm, while solitarious locusts displayed fewer straight trajectories and lower alignment.

Altogether our findings so far suggest the observed differences in group marching do not result from a change in simple visual processing or optic flow response. Instead, they highlight the importance of social interactions in driving locust collective marching behaviour.

Shaky affairs: Male vibratory courtship performance and mating success in the spider *Pisaura mirabilis* (Araneae)

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Communication is especially relevant in the context of reproduction and often implies complex courtship behavior. In spiders, substrate-borne signaling is particularly prominent and known to serve various purposes such as species recognition, reducing female aggression and transmitting information about male quality. Males of the Nursery Web Spider *Pisaura mirabilis* are well-known for offering a prey item wrapped in silk to females as a part of courtship behavior. In addition to this nuptial gift, males produce pre-copulatory vibrations by tremulating their opisthosoma and transferring pulses onto the substrate via their legs. There is a great variability in vibrations within a population of males with regard to pulse rate and the consistency of pulse intervals. However, a male's vibratory courtship performance appears to be individually consistent and can therefore be assumed to transfer honest information on his physical condition and genetic quality.

In this study we assessed the influence of the male vibratory courtship performance on female acceptance probability and mating success based on behavioral observations. After screening and ranking 117 *P. mirabilis* males in order of the quality of their vibratory performances, we staged 58 double-mating experiments. Each female was mated twice (in randomized order): once with a male classified as “high”-signaling and once with a male classified as “low”-signaling, allowing a direct comparison of both. During the trials, courtship and related behaviors such as tremulation, physical contact, leg rubbing, gift-wrapping, gift acceptance, copulation, thanatosis and possible aggression were tracked. “High”-signaling males are expected to gain a fitness benefit via cryptic female choice expressed by shorter copulation latencies and longer copulation durations. The analysis of this data will evaluate if females show a preference towards “high”-signaling males in mating decisions.

When darkness exceeds contrast

Merit Meschenmoser, Volker Dür

Bielefeld University

Insects show robust visual orientation behaviour towards static objects even without net image shifts on the retina (e.g. Varjù, 1975). Recently, we have replicated findings on orientation towards black-and-white edges (e.g. Kalmus, 1937) and negative phototaxis in the absence of contrast edges (e.g. Zeng et al., 2020) in adult female stick insects (*Carausius morosus*). Our results show that, while luminance alone predicts heading direction, areas of high contrast (e.g. edges), if present, predict the final position of the animal. This suggests an interplay of both phototactic and edge orientation mechanisms.

Our aim was to further assess the relative contribution of negative phototaxis and edge orientation mechanisms during target choice behaviour. To this end, we designed a discrimination task in which animals were first cued to walk towards a gray bar and then offered an alternative target as a distractor. We used an adaptive staircase method to obtain gray level thresholds of the bar (with edges) at which the distractor (without edges) was preferred. As the animal was approaching the gray bar, a Gaussian luminance pattern (distractor) without edges, but with overall lower luminance than the bar, appeared to one side of the gray bar. Interspersed control trials used a gray bar of equal contrast and size as a distractor to test for a preference of initial direction.

We show that animals display a persistence of heading direction if the distractor was the same pattern. However, our results show that the initial approach of the gray bar, could be redirected towards the Gaussian distractor, if the contrast of the gray bar was reduced by more than 15 percent. This suggests that phototactic mechanisms play an important role in cue conflict situations, even if the animal has a preference to orient towards edges.

***Saccopteryx bilineata*: A Promising Candidate for Exploring Neuronal Substrates of Vocal Learning**

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The Greater sac-winged bat *Saccopteryx bilineata* is a unique mammal with remarkable features, including vocal learning, vocal practice, and song. The learning and practice of syllables are manifested in the production of long multisyllabic vocal sequences, called pup babbling. This bat species is the only mammalian vocal learner besides human infants known to exhibit a babbling phase during vocal ontogeny.

I present my current project, which explores the neuronal substrates of vocal learning in *Saccopteryx bilineata*. Specifically, I aim to investigate the functional role of speech-relevant genes, such as FoxP2, during vocal learning. I hypothesize that I will observe an upregulation of FoxP2 during babbling (i.e. ongoing vocal learning processes) compared to my control groups (i.e. silence and innate vocalizations).

Furthermore, I am examining the expression of immediate early genes to map neuronal activity during vocal practice and learning.

To accomplish this project, I have successfully implemented a synergy of lab and field methods by combining behavioral observations and acoustic recordings of wild pups with subsequent harvesting of brains in the field (perfusion method). Currently, I am analyzing the expression of the different genes with the method of free-floating in-situ hybridization. The ultimate goal of this project is to investigate speech-relevant genes in a vocal learning mammal and establish connections between findings from songbirds and humans.

Insights into novel object tests

Sabine Kraus

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A widely used method to study the personality of animals is the novel object test. It is assumed that the shorter the latency to approach an unfamiliar object, the less neophobic the animal. This implies a fear response to the novel object, while it usually does not take into account whether the unfamiliar object triggers a motivation to explore.

Novel object tests often differ in both methodology and interpretation of the behaviours measured. To get a deeper insight into what is actually measured with the novel object test, we have performed different versions of this test with zebra finches (*Taeniopygia guttata*). We repeatedly performed novel object tests in a familiar and an unfamiliar environment. Besides the behaviour towards the unfamiliar object, we also measured the activity of the animals during the tests. In addition, we took blood samples to determine the plasma concentration of corticosterone, the most important stress hormone in birds.

An increase in corticosterone in combination with aversion to the new object would indicate that birds are neophobic. Our results show that zebra finches hesitate to approach a new object, particularly when they are in an unfamiliar environment. We found little variation or repeatability of behaviour towards the new object in both situations. However, there were different patterns of activity. Moreover, we found a clear avoidance of preferred water baths in combination with increased stress hormone concentrations in the presence of a new object, suggesting that the birds were neophobic rather than showing a lack of interest in exploration.

Taken together, our data provide a detailed overview of zebra finch behaviour towards novel objects.

Morphology and behavioural use of silk glands in spiders: a case study of *Pholcus phalangioides* (Araneae: Pholcidae)

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Spiders have the remarkable ability to produce different types of silk from multiple silk glands, but our knowledge about how they utilize these silks and the functions of the various glands is limited. In this study, we focused on the cosmopolitan spider species *Pholcus phalangioides*, colloquially referred to as Daddy long-legs spiders. These spiders construct irregular three-dimensional webs consisting of anchor points for web attachment, tangle webs for capturing airborne prey, and viscous-sticky traps to ensnare ground-dwelling prey. To comprehend the diversity of silk types, silk gland utilization, and properties, we collected diverse silk samples from *P. phalangioides* webs in a controlled laboratory environment for microscopy and mechanical analysis. We identified three distinct types of silk fibres based on variations in fibre diameter within the collected samples, relating to the different diameters of spigot pores of the different gland types. By dissecting the spinning apparatus of both male and female *P. phalangioides*, we studied the morphology of the silk glands, comprising one pair of major ampullate glands, one pair of minor ampullate glands, one pair of aciniform glands, one pair of large glue glands, and 4-5 pairs of small glue glands. Utilizing Raman spectroscopy, we compared the chemical profiles of glue secretions produced in different situations, revealing similarities between the glue used in prey wraps and anchor points, while the viscid glue obtained from the gumfoot tangle showed different profiles. For fibre samples, we found the differential inclusion of major ampullate, minor ampullate and aciniform silk as well as different number of fibres for different silk products such as the dragline, web sheet, tangle lines, gumfoots, prey wrap and egg sac. This is the first step towards a comprehensive understanding of the ecological role of silk gland differentiation spiders beyond orb-weavers.

The Arachnid mind: Insights into embryonic brain development in *Parasteatoda tepidariorum*

Jessica Kolbe

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Spiders have evolved a huge variety of locomotion and prey capture methods and associated behavioural biology. Little is known how this diversity is reflected in the morphology and development of the central nervous system, especially the brain. The components of the central nervous system of adult spiders are intimately fused and are mainly concentrated in the prosoma. However, during embryonic development the segmental primordia, including those of the anterior brain region and of the opisthosomal segments, are still well separated and can be studied in more detail.

Limited research has been done on the embryonic and postembryonic brain in our model spider species *Parasteatoda tepidariorum*. Studies of several key factors of head allocation and head segment development have already shown, that anterior development uses some conserved mechanisms that are also known from other arthropod species, but there are few detailed studies of brain morphology and development of embryonic and postembryonic stages. Therefore, I would like to study embryonic brain development in *P. tepidariorum*, at the morphological as well as at the molecular genetic levels.

The aim of the project is to identify molecular genetic factors that influence brain development and its specific substructure. Does a divergent structuring of the brain/CNS already take place in the embryos of different spider species? For this purpose, candidate genes/marker genes known in the model organism *Drosophila melanogaster* to be involved in the formation of brain anatomy will be studied in *P. tepidariorum*. Using molecular cloning, in situ hybridisation, immunocytochemistry and histological sections (with staining), candidate genes and their role in brain development will be studied. Parental RNAi will be applied to examine whether morphological alterations occur in the anatomy of the brain and possibly lead to changes in certain locomotory capabilities of the animals.

Timecourse transcriptomics to decipher molecular periodicity of *Clunio marinus*' circalunar clock

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Biological clocks allow organisms to adjust their physiology and behavior to regular environmental cycles. The marine non-biting midge *Clunio marinus* (Diptera, Chironomidae) inhabits the intertidal zone along the European Atlantic coast. Most of the insect's life cycle is spent as larvae that live on the sea floor, while adults are very short lived and require exposed algal substrate for oviposition. The algal substrate is most consistently exposed during the lowest tides, which occur around full moon and new moon. Thus, the emergence and reproduction of *C. marinus* adults is controlled by a circalunar clock to take place at these times. Most known populations of *C. marinus* emerge periodically either at full moon or new moon, or at both full moon and new moon. However, some populations emerge arrhythmically on any day of the lunar cycle. The molecular components of the circalunar clock and the basis of the differences in adult emergence of the different populations are largely unknown.

We employ RNA-seq to investigate the rhythmically expressed genes throughout the lunar cycle in LIII larvae of three *C. marinus* populations exhibiting different emergence patterns, namely emergence during full moon, emergence during new moon or arrhythmic emergence. Rhythmically expressed genes could represent circalunar clock components themselves and/or their potential targets. We also assess the genomic environment around the identified genes to explore their upstream control. Together this sheds light on the transcriptomic basis of the differences in adult emergence in these populations and helps elucidate the molecular underpinnings of *C. marinus*' circalunar clock.

Gene and genome duplication and phenotypic novelties – Insights from spiders

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Phenotypic novelties play a crucial role in enabling organisms to adapt more effectively to their environment, thus driving the process of evolutionary diversification. Gene or genome duplications at either small or large scales have been linked to the emergence of such novelties. When a gene is duplicated, one of the copies may undergo changes over time, acquiring new functions that are beneficial in a specific environment. While vertebrates underwent multiple whole genome duplication events, our understanding of these processes in arthropods, such as spiders and scorpions, has remained limited. Fortunately, a recent study has shed light on a significant gene duplication event that occurred in the common ancestor of arachnoplumonates (spiders and scorpions). This finding is particularly intriguing because spiders possess distinctive phenotypic novelties like specialized breathing organs and silk glands. Therefore, spiders serve as excellent models for investigating the mechanisms underlying diversification of gene function after duplication. Here, we study the gene content of different spider species to reveal species-specific gene duplication events that contribute to their diversity. To unravel the mechanisms underlying the diversification of regulation and expression of duplicated genes, we will explore temporal expression patterns and chromatin accessibility during embryonic development in three species. Moreover, we will assess transposable element content in various spider species. For a selected group of gene duplicates that show diversified expression, their functions will be experimentally validated using techniques like RNA interference-mediated gene knock-down. Through this research, we aim to provide novel insights into the extent, nature, and consequences of gene and genome duplications. Furthermore, we seek to understand their significance in driving the evolution and diversification of phenotypic novelties.

Developmental and molecular mechanisms underlying the formation of head horns in the broad horned beetle *Gnathocerus cornutus*

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Morphology is highly variable in nature and phenotypic novelties contribute to this diversity. A phenotypic novelty is a primary body plan, a new constructional element, or a newly individualized character, that is qualitatively discontinuous from the ancestral stage. Despite the importance of phenotypic novelties, the underlying genetic and molecular mechanisms are hardly understood. The broad-horned flour beetle *Gnathocerus cornutus* is a widespread pest of stored products and it features a phenotypic novelty in form of male-specific head morphology. In this sexually dimorphic species, male, but not female, beetles exhibit large mandibular horns, prominent genae and small horns on the vertex. In addition, its close relationship to the beetle model *Tribolium castaneum* offers developmental and genetic tools such as RNA interference. Therefore, this beetle is a tractable system to investigate the developmental and genetic processes underlying the formation of a phenotypic novelty. We show that the mandibular horns of *G. cornutus* develop during pre-pupal stages and we employed genome wide expression data for males and females to identify sexually differentially expressed candidate genes at these relevant stages of post-embryonic development. RNA interference experiments are being used to assess the function of these candidate genes during postembryonic stages, and we optimized a head clearing protocol to study head morphology at different developmental stages and upon gene knockdown using confocal laser scanning microscopy. Our results shed first light on the developmental and molecular mechanisms underlying novel head morphology in a beetle system.

Linking the cell cycle to membrane oscillations in *Drosophila* syncytial cleavage division cycles

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In *Drosophila melanogaster*, as in other insects, the first cell divisions during embryonic development occur without cytokinesis. At the beginning of the 10th division, the embryo enters the syncytial blastoderm stage, where most nuclei are located at the periphery of the embryo and continue to divide. To avoid interference of dividing nuclei, actin-rich membrane furrows move in between the spindles at the beginning of each prophase and retract during each telophase. The link between the cell cycle and cortical actin and membrane oscillations is unknown. The localization of actin regulatory proteins is coupled with membrane transport through the recycling endosome (RE). RE transport requires Rab11, a small GTPase and Nuclear Fallout (Nuf), a Rab11 effector and adaptor protein to the Dynein microtubule motor. This transport mechanism is responsible for membrane growth during cellularization at cycle 14. We found that the gene drop out (dop) encoding the single homologue of human MAST kinases, is required for this membrane growth. In a quantitative proteomic approach, we identified the serine 401 residue of dynein-light intermediate chain (Dlic) as potential substrate of Dop. Overexpression of Rab11, Nuf or phosphomimetic variants of Dlic suppressed membrane growth defects in dop mutant embryos. This led us to a model in which the phosphorylation of Dlic-Ser401 promotes its interaction with Nuf to control Rab11 dependent transport. This phosphorylation may also regulate Nuf-dependent transport of actin activators during syncytial divisions. To test this possibility, a phosphoablative version of Dlic was overexpressed, which resulted in defective syncytial divisions. However, these divisions exhibited phenotypes resembling Nuf-related defects and spindle assembly checkpoint (SAC) abnormalities. Thus, the phosphorylation of Ser401 might not only be important for Nuf-dependent transport, but also for SAC silencing. We propose that the phosphorylation of Dlic is involved in linking the cell cycle with furrow formation during syncytial divisions.

The role of large-scale gene duplications in phenotypic diversity of spiders

Chetan Munegowda

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Spiders are a large group of animals with more than 51,000 identified species. They are very diverse in size, colouration, web-building behaviour, venom composition, silk production, and prey catching behaviour. Chromosome-level genome assemblies are only available for about 10 species. This lack of genomic data limits our understanding of the genetic underpinnings of the evolution and diversification of these fascinating eight-legged creatures. In our study we aim to generate chromosome level assemblies for spiders occupying interesting phylogenetic positions including the mygalomorph spiders (*Acanthoscurria geniculata* and *Ischnothele caudata*) and Mesothelae trapdoor spider (*Liphistius*).

Previous studies have suggested that a whole genome duplication event occurred in the common ancestor of spiders and scorpions. They have also speculated that multiple duplication events took place in their descendants. Gene duplication events provide a source for new gene functions which might lead to the development of morphological novelties. Several studies also show that gene duplications in spiders have contributed to phenotypic novelties like the diversification of venom arsenal, varying silk compositions, as well as developmental processes like leg-patterning. Using the chromosome level genomes, we will systematically identify duplicate and single copy genes common for all spiders, as well as those of specific lineages. Comparative analyses across arthropods will allow us to test whether these duplications may also be involved in the evolution of phenotypic novelties.

Into the shadows: Molting in the common house spider *Parasteatoda tepidariorum*

Denise Klinkenbuß

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Molting controlled by ecdysteroid hormones is a key process in the lifecycle of all ecdysozoans, and is essential for growth or metamorphosis. In both *Pancrustacea* (e.g. Insects, Crustaceans) and Arachnida, the 'Halloween genes' (HG) are conserved components of the ecdysteroid synthesis pathway. HGs encode cytochrome P450 (CYP) enzymes and, along with other genes involved, are expressed in the prothoracic gland of *Drosophila* or the Y-organ of crustaceans.

To study the role of the HGs in the spider model organism *Parasteatoda tepidariorum*, the mRNA expression of the HG homolog shadow was studied using WISH on late-stage embryos and vibratome sections of subadults. Knockdown experiments with parental RNAi were performed to establish the function of shadow in embryonic development. Additionally, subadult males were injected with shadow dsRNA to elucidate its function during postembryonic development and molting.

Expression of shadow shows a distinct pattern in a medial stripe close to the dorsal opisthosoma surface, which resembles the migrating pericardial cells during dorsal closure, suggesting a possible role in heart development. In addition, gene expression is detected in a symmetrical expression pattern in the developing brain of *P. tepidariorum*.

Knockdown of shadow resulted in increased (early-stage) embryonic lethality or fatal molting complications in hatchlings during the first ecdysis. Surviving spiders showed delayed molting cycles and remained smaller than the control animals. Likewise, the phenotype of injected subadult males included delayed molting cycles and incomplete molts.

The observed molting defects in RNAi knockdown animals demonstrate that at least shadow is involved in spider molting and suggests a conserved role of shadow in the spider ecdysteroid synthesis pathway.

Germ-disc formation in spiders – a stochastic process?

Matthias Pechmann

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Early spider embryogenesis involves the formation of a radially symmetric germ-disc. As the cells of the rim of this germ-disc will develop into anterior structures like the head, the centre of the disc will form the posteriorly located segment addition zone of the embryo. Therefore, germ-disc formation sets the anterior posterior body axis of the spider embryo. The early spider egg is a spherical structure with no apparent asymmetry and it is believed that germ-disc formation is a stochastic process.

Here we provide first evidence that germ-disc formation in the common house spider *Parasteatoda tepidariorum* is not a stochastic but pre-determined process. Furthermore, we show that this predetermined process might be conserved throughout different spider orders. Our analyses provide a good starting point to better understand axis formation in spiders in future studies.

Kill it with venom – Embryonic development of venom glands in the spider *Parasteatoda tepidariorum*

Franziska Wöhrmann-Zipf

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Kill it with venom – Embryonic development of venom glands in the spider *Parasteatoda tepidariorum*

Spiders are a morphologically diverse group of arthropods, adapted to a wide range of habitats and lifestyles. One reason for their evolutionary success is their prey-hunting strategy - they incapacitate or kill their prey with venom. The venoms are often of interest because of their composition and potential application in medicine. Recently, interest has focused on the venom producing organ - the venom gland. The origin and relationships of spider venom glands are currently the subject of controversy. Spider venom glands are hypothesized to have evolved from salivary or silk glands precursors in early chelicerates.

The spider *Parasteatoda tepidariorum* will be used to study the embryonic origin and postembryonic development of the venom glands in more detail. Comparisons with other glands, e.g. silk glands, will reveal developmental and histological similarities or differences, in order to assess the possible serial homologies and evolutionary origin of the venom glands. To gain a better understanding of gland morphology and development of venom glands, high-resolution nano-CT scans will be used to construct a 3D model across all developmental stages. In addition, homologs of genes with known functions in e.g. salivary gland development in insects will be isolated from *P. tepidariorum*. The expression of these candidate genes in the spider will be studied on whole body preparations and vibratome sections. Furthermore, the potential role of the candidate genes in venom gland development and function will be determined by knockdown of genes with parental RNAi.

These studies will reveal the allocation and further development of the venom gland in spiders and will provide insights into both serial homology of venom glands with other spider glandular structures and direct homology with similar gland types in other arthropods.

Sense organ development in spiders

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Spiders possess a great diversity of sense organs. External sense organs are essential to perceive various sensory modalities and are thus also required for spider specific evolutionary novelties, like silk production, interspecies communication and prey capture using the web. 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, especially on the appendages. Contrasting their great importance and diversity the genetic control of sense organ development is only studied in a handful of arthropod species, mostly insects. The aim of this study is to reveal the genetic basis of spider sense organ development. By investigating sense organ development in *Parasteatoda tepidariorum* using in situ hybridization, we found that the transcription factor families Achaete-Scute-Complex (Pt-ASH1 & 2), Atonal (Pt-ATO1 & 2, and PAX (Pt-Pax 2.1 & 2.2) are duplicated and some of the paralogs are expressed as proneural clusters in the developing 1st legs. In contrast to findings in *Drosophila* the different genes show overlapping expression especially in the medial and distal leg segments. In the next steps we want to define the spatial and temporal co-expression of these genes in developing appendages and test their proneural potential via RNAi mediated knockdown. Interestingly, Pax2.1 is the first gene of the retinal determination network to be expressed early in the primordia of all lateral eyes in more than one spider species. Usually Pax6 is required for initiation of eye development but in chelicerates it is only expressed very late during head development. Using a combination of different functional tools we also plan to test the role of Pax2.1 in eye development.

Role of the developing olfactory epithelium in face morphogenesis: insights from scRNA-seq

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Interactions among different cell lineages play an essential role in craniofacial morphogenesis. The facial mesenchyme, derived from the cranial neural crest and the mesoderm, give rise to skeletal tissues such as cartilage and bone, that ultimately shape the face. Signaling centers located in other embryonic structures, such as the ectoderm and the developing brain, provide precise spatiotemporal instructive cues that coordinate the patterning of the facial mesenchyme, and in turn of the facial skeleton. Previous studies from our group showed that signals from another embryonic structure, the developing olfactory epithelium, play a critical role in face morphogenesis. Specifically, non-neuronal cells of the olfactory epithelium were hypothesized to be the source of these instructive signals. However, the molecular make-up and developmental trajectories of these non-neuronal cell types remain unclear.

In order to infer these molecular interactions and the olfactory cell types involved, we performed single-cell transcriptomics on the early murine developing face. We recovered all relevant cell types including the facial mesenchyme, ectoderm, and the developing olfactory epithelium. From the later, we captured the entire diversity of cell, including the non-neuronal types from the onset of the respective lineages.

Our dataset provides new insights into the developmental dynamics of the olfactory epithelium at early embryonic stages, that has not been reported yet. We support these findings by RNA velocity analysis and experimental validation using specific marker genes. Genetic lineage tracing will provide further evidence of cell type origin and differentiation. This study is instrumental for identifying the cell types and molecular signals coming from the olfactory epithelium that are essential for face morphogenesis. The role of these cells and molecules is being tested using genetic manipulation approaches.

Movement and foraging ecology of the Antarctic's smallest breeding birds

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Many species of seabirds are experiencing population declines, which in the future are expected to continue or even accelerate. With a fundamental place in the marine food web, both as prey and predators, seabirds form an integral part of marine ecosystems. Since seabirds also are exceptionally susceptible to changes in marine environments, e.g. effects of the ongoing climate change and anthropogenic pressures, they may function as monitors of changes in the marine ecosystem, such as shifts in the abundance of prey species. Storm-petrels (Hydrobatidae and Oceanitidae, order Procellariiformes) are among the most abundant pelagic seabirds worldwide. However, because of their pelagic lifestyle and small size, their trophic ecology is difficult to study, particularly so for species nesting in hard-to-reach remote areas in cracks and crevices such as some of the smallest members of the Procellariiformes breeding sympatrically in Antarctica: the Wilson's storm-petrel, *Oceanites oceanicus* and the Black-bellied storm-petrel *Fregetta tropica*. Using different approaches (baseline-independent stable isotope analyses, GPS and GLS tracking, metabarcoding of diet contents from faecal and regurgitate samples, and breeding parameter data) our project aims to analyse the foraging and movement ecology of these two storm-petrel species during their breeding season on King George Island, Antarctica, but also during their non-breeding period which they spend on the open ocean. Along with a general overview of the project objectives, we present the first results (breeding parameters, foraging trips during chick-rearing period) of the breeding season 2023.

Suitability of exotic plants for wild bees

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Wild bees are essential pollinators of many angiosperm plants and often are highly specialized and dependent on specific plant genera (monolectic bees) or plant families (oligolectic bees). However, in public and private gardens, exotic plants are generally preferred over native plants, which are sometimes even considered as weeds. In addition, the use of these exotic plants in gardens is often considered as a measure against insect decline. Here we examined the suitability of native and exotic plants by comparing visitation rate of wild bee species. We studied pairs of one native and one exotic plant, selected based on their phylogenetic relationship, their secondary plant compounds, and their status as garden plant or weed. We expected more, especially monolectic and oligolectic bees, on native as compared to exotic plants. We will present the first results of this experiment.

Decoupling ingestion from respiration: How brackish mysids cope with thermal fluctuations

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Benthopelagic mysids play an important role in coastal ecosystems. The mysids can experience high thermal variability on short time scales particularly in shallow waters, forcing them to permanently adjust their metabolism. Respiration rates are directly influenced by temperature, whereas ingestion rates might additionally be influenced by behavioral aspects. We aim to understand how respiration and ingestion rates respond to temperature changes during a diurnal thermal fluctuation cycle and how both processes are linked. *Neomysis integer* was collected in a littoral zone of the Baltic Sea. After laboratory acclimation, mysids were exposed to either a constant temperature of 15°C or a daily temperature fluctuation of 15±5°C. Short-term respiration and ingestion rates were measured at four equidistant time points within 24 hours. Under constant temperature, respiration and ingestion rates of mysids did not differ among time points. However, in the fluctuating treatment both rates showed significant differences among time points. Respiration was highest at the thermal maximum and lowest at the thermal minimum. Ingestion rates showed the opposite pattern. When temperature transited the average, the direction of temperature change was crucial for the animals' response in respiration and ingestion rates. Our results suggest that even though respiration is instantaneously affected by temperature, it is additionally influenced by the previously experienced direction of thermal change. Ingestion appears to be decoupled from respiratory performance in likely stressful conditions.

Optimising High-throughput sequencing data analysis, from gene database selection to the analysis of compositional data: A case study on tropical soil nematodes

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HTS provides an efficient and cost-effective way to generate large amounts of sequence data, being a very powerful tool to characterize biodiversity of soil organisms. However, marker-based methods and the resulting datasets come with a range of challenges and disputes, including incomplete reference databases, controversial sequence similarity thresholds for delineating taxa, and downstream compositional data analysis. Here, we use HTS data from a soil nematode biodiversity experiment to address the following questions: (1) how the choice of reference database affects HTS data analysis, (2) whether the same ecological patterns are detected with ASV (100%) versus classical OTU (97%), and (3) how different data normalization methods affect the recovery of beta diversity patterns and identification of differentially abundant taxa. At this time, the SILVA database performed better than PR2, assigning more reads to family level and providing higher phylogenetic resolution. ASV- and OTU-based alpha and beta diversity of nematodes correlated closely, indicating that OTU-based studies represent useful reference points. Further, our results indicate that rarefaction-based methods are more vulnerable to missed findings, while clr-transformation based methods may overestimate tested effects. ANCOM-BC retains all data and accounts for uneven sampling fractions for each sample, suggesting that this is currently the optimal method to analyze compositional data. Overall, our study highlights the importance of comparing and selecting taxonomic reference databases before data analyses, and provides solid evidence for the similarity and comparability between OTU- and ASV-based nematode studies. Further, the results highlight the potential weakness of rarefaction-based and clr-transformation based methods. We recommend future studies use ASV and that both the taxonomic reference databases and normalization strategies are carefully tested and selected before analyzing the data.

Swimming in ‘Stealth Mode’? Influence of Predator Presence on the Fluid Mechanics of *Daphnia longicephala* and its Ecological Consequences

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Daphnids are of central importance in limnic ecosystems worldwide, as they link the producers’ with the consumers’ levels. They are prey for many different predators and have evolved inducible defences that make them less susceptible to predation. Since morphological alterations are only observed when predators are present, it is assumed that they increase the individual’s and/or the populations’ fitness.

However, the defensive mechanisms of the morphological alterations are still unknown. It is hypothesized that predators perceive the water disturbances elicited by their prey to follow and capture them. A different morphology might therefore cause a beneficial fluid flow around the daphnids that disguises their real position.

We investigated the influence of an enlarged crest on the fluid flow of *D. longicephala*. A static simulation showed a beneficial water flow around the defended morph, as it reduces conspicuous flow patterns. With particle image velocimetry we found that the water movement that results from the antennal stroke during the active locomotion superimposes the possible fluid mechanical alterations caused by the defended morphology. Overall, conspicuous water displacement dissipates in the vicinity of the locomoting daphnid and is not observed in distances larger than one body length. Nevertheless, predation trials again revealed a higher survival rate for defended in comparison to undefended *D. longicephala* due to enlarged handling times.

Thus, the morphological defence does not contribute to a disguise during swimming. It seems unlikely that predators are able to detect their prey by noticing the water displacement. Further research is necessary on both sides of the predator–prey–interaction to reveal the function and mechanisms of induced morphological alterations in daphnids and how predators are thwarted. Future work can therefore focus on other signals that daphnid swimming might elicit, for example in the context of pressure propagation or underwater acoustics.

Nematode *Panagrellus redivivoides* affects *Drosophila melanogaster*-microbiota interactions

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Insects are recognized for hosting microbiota and forming symbiotic partnerships with them. However, these interspecific interactions are not limited to microbiota alone in some insect species. Quite frequently, non-parasitic nematodes – also known as roundworms – are found cohabitating with insects. This combination of micro- and macrobiota has the potential to influence crucial aspects of the insects' life cycle and, consequently, their developmental success.

The impact and the role of non-parasitic nematodes on the ecological success of insects largely remains unknown. Here we take a look at the effect of a cohabitating nematode species, *Panagrellus redivivoides*, on *Drosophila melanogaster* and their associated microorganisms in different habitat patches.

Following the discovery of nematodes in microcosms harboring *D. melanogaster* population at semi-natural breeding conditions, we successfully have identification the species as *P. redivivoides* and delved into the reciprocal interactions between *P. redivivoides* and the life cycle of *D. melanogaster*. Upon observing that the adult flies can serve as a vector for the nematodes, helping the worms colonize new habitat patches, we found that rapidly growing nematode populations suppress the development of filamentous mold fungi, raising the question of how nematodes may influence insect development through manipulation of microbiota. To address this question, we exposed insect larvae and nematodes to various combinations of fruit types and fly-fecal microbiota as breeding sites and observed the fitness outcome of the fly larvae.

First, we looked at a single substrate environment based on apple and evaluated larval fitness - i.e., weight of emerging flies, developmental time-, which improved when nematodes were present. However, this effect seems to be variable as it could not be reproduced in other substrate environments - lemon and tomato. Based on these results, we speculate that the environment-dependent outcome of *Drosophila-Panagrellus* interactions prevents the evolution of specific associations to develop between them.

A Heated Exchange: Unraveling the Impact of Heat Stress on Fruit Fly Symbiosis

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Fruit flies (*Drosophila*) are ecologically successful insects, adapted to thrive on many ephemeral plant tissue. These substrates naturally harbor microbial communities that play a crucial role in the fly life cycle, providing nutrients but also excreting toxins that can influence larval development. Fruit flies can enhance the success of their offspring by transmitting their own symbiotic microbes during egg laying. Despite fruit flies being widely used as model systems to study insect microbiomes, our understanding of these interactions in their natural environment remains limited. Especially with the prospect of climate change and now often occurring temperature extremes, even less is known how this environmental pressure would affect the seemingly successful symbiosis. We investigated the impact of heat on the *Drosophila* life cycle by simulating heat waves (30-60-90-120 minutes at 40°C) on larva-microbe-substrate microcosms. To disentangle the effects of temperature extremes on *Drosophila* larvae and their associated microbes, we conducted three consecutive experiments. First experiment subjected the larvae to a heat wave before introducing them onto a natural substrate inoculated with their parental symbiotic microbiota, observing a response with increased development time and mortality, but larger adults. In the second experiment we induced a single heat wave (40°C for 1 hour) on larva-microbe-substrate microcosms at different developmental stages of *D. melanogaster*. Surprisingly, we found that 3rd instar larvae were the most susceptible to heat wave, resulting in highest mortality (80%). In the follow up experiment we induced a heat wave on inoculated substrate before adding the larvae and included both native and mismatched microbiota from a different substrate. We observed that heated native microbiota promoted faster development in flies, while mismatched microbiota slowed down their development. Overall, these findings emphasize the largely overlooked role of interactive effects of microbial communities and increasing frequency of temperature extremes on insect development.

Function of insectivorous songbirds in forest ecosystems - An analysis along the trophic cascade bird-caterpillar-tree

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In recent years, global warming significantly impacts forest ecosystems in the temperate climate of central Europe. Frequent summer droughts and infestations of insect pests, such as herbivorous caterpillars, stress the resilience even of well adapted forest species. Declining biodiversity can further unbalance these ecosystems.

In this context, this study aims to investigate the regulating function of insectivorous songbirds in forest ecosystems whose main diet during the breeding season is herbivorous caterpillars.

Therefore, the following questions are addressed, (i) to what extent the abundance of insectivorous songbirds can be artificially increased, (ii) whether a change in bird abundance affects caterpillar biomass, and (iii) whether a change in caterpillar biomass leads to less damage to trees in the form of leaf browsing, which is then reflected in tree growth rates.

Two study plots each were established in two deciduous tree-dominated forest areas (beech and oak), once in an area with nest boxes (n=147) and once without nest boxes. In these areas, bird abundance and density were documented using breeding bird mapping, and caterpillar biomass as well as leaf browsing was quantified and evaluated using statistical methods, including Generalized Additive Models (GAMs), in two seasons.

Results confirmed significantly increased bird density in areas equipped with nest boxes.

On average, the nest box sites revealed a reduction of caterpillar abundance by 32% (2021) and 52% (2022) compared to the control sites. There was also less (44%) caterpillar induced leave damage, estimated as the reduction in leaf area from canopy branches in 2022.

In summary, the results indicate that insectivorous birds provide a regulatory function in forest ecosystems. Monitoring is continued during the upcoming season. If our results are confirmed, maintenance and cultivation of bird populations should be acknowledged as a key aspect of ecosystem stability and forest management.

The molecular signatures of adaptation to different latitudes in seminal fluid proteins of *Drosophila melanogaster*

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Male ejaculate contains seminal fluid proteins and peptides (Sfps) that play a crucial role in reproduction and vary widely among species. So far, research has largely focused on their rapid evolution due to post-mating selective forces. However, little is known about the influence of abiotic factors e.g. temperature on the rapid evolution of Sfps. We, here, investigated the molecular signatures of selection and population differentiation in Sfps among different latitudes and continents in *Drosophila melanogaster* using a global SNPs dataset. Our preliminary results show that several genes encoding proteins involved in heat stress and immunity show a correlation between the intensity of selection and latitude. Furthermore, the study suggests that there are local adaptations in seminal fluid that are due to geographical location, as each continent has a different list of genes that show such relationships.

Detecting Wolbachia infection with Real-Time-PCR in female-biased flea beetle, *Altica lythri*

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Wolbachia (Rickettsiales) is an intracellular pathogen that infects many arthropods and has become famous due to its ability to manipulate the host's reproduction. In the flea beetle *Altica lythri* a statistical correlation between different Wolbachia strains and *A. lythri*'s mitochondrial DNA (mtDNA) haplotypes has been established. A strongly female-biased sex ratio distortion was consistently observed in beetles with mtDNA HT1 or a slight variant of HT1, called HT1*. The latter is in general not infected with Wolbachia, while beetles with HT1 showed consistent presence of Wolbachia infection. The mtDNA HT1 beetles produce exclusively female offspring which is also true for the usually uninfected HT1* beetles. At first a Wolbachia infection of the strain wA1 found in HT1 was considered to be a potential cause for the female bias. However, the mostly uninfected HT1* beetles also consist only of females.

Recently, it was shown that Wolbachia infection rates are widely underestimated in different taxa. Likewise, pool sequencing of *A. lythri* haplotypes has confirmed some infections among HT1* beetles and dispelled the invulnerable myths of this haplotype. The reason is that the low level of the infection was hardly detectable with previous methods such as normal Taq-PCR. Thus, we establish a novel Real-Time-PCR assay to detect and quantify these low-level infections. It turns out that HT1* beetles are not completely uninfected as previous research suggested, but have infection levels significantly lower than the other haplotypes. Apart from this, some unexpected combinations of Wolbachia strain and mtDNA haplotype (e.g. HT1* with wA2 instead of wA1) were found. This could potentially be used to obtain insight into how Wolbachia is transmitted horizontally intra- or even interspecifically and explain why over the last years a double infection of wA2 and wB has become dominant in HT2 beetles of our focus population.

Excretion and Reabsorption of Cardenolides in the Malpighian Tubules of the large milkweed bug *Oncopeltus fasciatus*

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In the course of co-evolutionary interactions between plants and herbivorous insects, plants evolved defense strategies to avoid feeding damage. Cardenolides are an important group of toxic plant secondary metabolites that block the NA,K-ATPase of herbivores. However, several insects have evolved the ability to tolerate them and even store and secrete them as a protection against their predators. The large milk bug (*Oncopeltus fasciatus*) for example is able to actively accumulate cardenolides in storage compartments from where they are released as a defensive secretions.

Studies on cardenolide-tolerant leaf beetles of the genus *Chrysomela* suggest that cardenolides are actively reabsorbed from Malpighian tubules into the haemolymph, probably catalysed by membrane-associated transporter proteins. ABC proteins of subfamily B (ABCB) likely play an important role in this process, as previous experiments with *O. fasciatus* showed a strong accumulation of ABCB transporters in cellular residues within the defense secretions. Bioinformatic analyses of the transcriptome resulted in four ABCB full transporters and five half transporters. Quantitative real-time PCR revealed the tissue-specific expression profile, supporting the hypothesis that the Malpighian tubules play a central role in the cardenolide transport pathway in *O. fasciatus*.

In subsequent studies, the excretion and reabsorption of cardenolides in the Malpighian tubules will be determined in different phenotypes of *O. fasciatus* using a Ramsay assay. In this physiological assay Malpighian tubules are isolated and stretched across a water-mineral oil interface: The distal end of the tubules is in an aqueous environment containing cardenolides, while the proximal end is in mineral oil. In this setup, the uptake of cardenolides at the distal end into the Malpighian tubules and the reabsorption at the proximal end into the mineral oil can be studied. To verify the ABCB-associated cardenolide transport, phenotypes are generated in which the translation of a specific ABCB transporter is silenced by RNAi.

Stability versus flexibility: phenotypic robustness differs between queen and worker caste

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The reproductive division of labor in social insects, with a reproductive queen and specialized workers for colony maintenance, shows parallels to the differentiation of germline and soma in multicellular organisms (Weismann, 1893). Somatic cells have evolved a stunning phenotypic diversity and functional specialization, providing the foundation for complex body plans. In analogy, social insects have a wide variety of worker phenotypes with morphological, physiological and behavioral adaptations and specializations, both temporal and permanent, thus allowing the exploitation of a huge range of habitats and niches. Relaxed evolutionary constraints in workers due to indirect selection is one factor underlying the apparently faster trait evolution in this caste. Previous work found that workers of the invasive ant species *Cardiocondyla obscurior* have a lower phenotypic robustness (i.e. less stringent phenotypic canalization) compared to queens when simulating stressful/aberrant environmental conditions via pharmacological suppression of Hsp90, a central player of the cellular canalization machinery. It was further shown that queens show a faster and more intense regulatory answer to our treatment. Hence, analogous to the germ-plasm theory, queens (germline) seem to have a higher level of protection against environmental disturbances potentially leading to phenotypic variation than workers (soma). We aim to elucidate the causal relationship between gene expression patterns and phenotypic robustness, comparing transcriptomes of queens and workers, whose stress-response have been challenged.

Our research will contribute to a broader understanding of the functional and developmental consequences of the differentiation between germline-soma in multicellular organisms and queens-worker in social insects.

Novel task for a novel caste – Controlling microbial growth

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The gut microbiome of ants contributes to their metabolism and immunity. Controlling the composition of the gut microbiome is essential for the host to avoid overgrowth of harmful microorganisms and their spread through the whole colony. *Myrmecocystus mendax* belongs to an ant genus which evolved a special worker caste, called repletes or honeypots. Healthy honeypots have enlarged crops and gasters filled with a nutrient-rich liquid, which can be dispensed to the colony if required. Besides the capacity to enlarge their abdomen, honeypots need mechanisms to conserve their gut content and prevent unwanted microbial growth. We repeatedly found that honeypots in isolated worker groups accumulate gas in their crop and often die.

We hypothesized, that the variation between healthy and gas-filled honeypots is due to a dysbiosis of their crop microbiome. This was supported by microscopic analyses of the crop liquid, which showed that yeast-like cells were abundant in gas-filled honeypots but almost absent in healthy honeypots. By using a cultivation-dependent approach, several colonies of the yeast *Zygosaccharomyces bailii* were isolated from the gas-filled honeypots, while bacteria from the genus *Fructilactobacillus fructivorans* were isolated from both honeypot variants. This suggests, that growth of the yeast *Zygosaccharomyces bailii* can lead to a dysbiosis of the crop microbiome of *Myrmecocystus mendax*, with adverse effects on the survival of the repletes.

We will analyze the crop microbiome of both honeypot variants using 16S amplicon sequencing. Therefore, we will compare these microbiomes with those of other *Myrmecocystus mendax* worker castes and its sister genus, *Lasius niger*. In combination with RNAseq analyses we want to understand the underlying mechanisms that help to preserve the crop content of honeypots and hence the necessary physiological and evolutionary changes associated with the evolution of honeypots as a new worker caste in the ant genus *Myrmecocystus*.

Ionotropic receptor genes in *Daphnia magna*

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Ionotropic receptors (IRs) are encoded by a relatively newly discovered and diverse group of sensory receptor genes discussed to have evolved from ionotropic glutamate receptors (iGlurs) - a highly conserved family of ligand-gated ion channels. First described in *Drosophila melanogaster* some conserved homologues are present among protostomes, with clade-specific expansions.

IRs are heterotetrameric complexes of broadly expressed co-receptors and functionality-specific expressed tuning receptors. These are involved in different sensory modalities, such as olfaction, gustation, hygrosensation and thermosensation. While this gene family has been described to some extent in terrestrial arthropods, it is only vaguely or not at all described in aquatic species with their sensory modality mostly undetermined.

In the microcrustacean *Daphnia pulex* genome, 85 IRs have been found by hidden Markov modelling but were not annotated further. Similarly, in *D. magna* newly sequenced and more extensive annotated genomes delivered annotated gene models. However, these annotations and the *D. pulex* IR sequences are derived from automated pipelines and without gene model verification. This is mandatory when studying gene function e.g. using genetic screening or in heterologous expression.

In the current study we aimed to identify and annotate the IR genes in the *Daphnia magna* genome and subsequently performed a preliminary phylogenetic analysis. We used the *D. pulex* IR set as an initial query and performed a homology-based iterative tBLASTn approach. By this we curated and if necessary, corrected the identified genes manually and verified them with RNA-seq data and the protein domain prediction tools DeepTMHMM and SignalP6. We obtained a set of 79 putative *D. magna* IR genes which were analysed phylogenetically together with *D. pulex* and *Drosophila melanogaster* sequences.

This new set of IR genes from the curated annotations will now serve as the basis for further evolutionary and functional studies of this novel chemoreceptor gene family.

ABCB transporters of *Oncopeltus fasciatus* – Functional importance and substrate specificity

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Milkweed bugs of the species *Oncopeltus fasciatus* (Heteroptera, Lygaeidae) feed on plants of the genus *Asclepias* (Apocynaceae) which contain toxic cardenolides. The bugs evolved intricate adaptations to tolerate and detoxify these compounds. Their ability to tolerate the plants compounds is paralleled by a general propensity of insects to develop resistances to man-made insecticides. In both processes carrier proteins – ABCB (ATP binding cassette) transmembrane transporters play an important role as they transport xenobiotic substances and act as efflux transporters in the blood brain barrier. A better understanding of ABCB transporters, their functional roles and the structures determining their substrate specificity is thus essential both from an evolutionary point of view and in the light of applied aspects. We identified two ABCB-transporters, ABCB1 and 2 in the transcriptome of *O.fasciatus* and cloned them into expression vectors. By using baculovirus infection of insect cells we achieved the heterologous expression of these proteins. In order to investigate their function we use enzyme assays of inverted membrane vesicles isolated from these cells. The activity of the ABCB-transporters can then be measured by the rate of ATP hydrolysis revealed by the released phosphate. In this way we here compare the specificity of the two ABCB transporters for various substrates.

Evolution of Globin Proteins in Vertebrates: Insights into the Transition from Water to Land (The Rise of Tetrapods)

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The transition from water to land during the rise of tetrapods represents a significant milestone in vertebrate evolution. Understanding the underlying molecular mechanisms that facilitated this transition is of great scientific interest. One critical aspect of this transition is the evolution of globin proteins, which among other things, play a crucial role in oxygen transport and storage. In this study, we investigate the evolutionary changes in globin proteins, focusing on vertebrate lineages and their relevance to the water-to-land transition.

The most well-known globins are hemoglobins, responsible for oxygen transport in red blood cells. However, vertebrates have also been found to possess other globin variants, including myoglobins, neuroglobins, cytoglobins, globin E, globin X, globin Y, and androglobin, each exhibiting unique functions in specific tissues. Besides their role in oxygen transport, globins have been shown to scavenge nitric oxide, provide oxidative stress protection, participate in gas sensing, perform nitrite reduction, and exhibit enzymatic activities such as peroxidase or oxidoreductase functions.

Our findings reveal lineage-specific duplications and losses of globin genes, suggesting dynamic evolutionary events. Teleost fishes exhibit a rich diversity of globin isoforms, potentially contributing to their adaptation to different aquatic environments. Tetrapods display significant alterations in globin gene expression and protein structure compared to their aquatic ancestors. This includes changes in oxygen affinity and regulation, enabling efficient oxygen extraction from air.

Overall, our study highlights the importance of globin proteins in understanding the evolutionary transition from water to land. Further investigations into the functional properties of different vertebrate lineages will deepen our understanding of this crucial evolutionary event and the mechanisms underlying the rise of tetrapods.

Are Older Siblings Better Siblings?

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Family life is a widespread phenomenon in the animal kingdom and can be found in many taxa. Family life entails multifaceted interactions between family members. Sibling cooperation, for example, is a key feature of eusociality. Sibling cooperation can also be found in e.g. cooperative breeding birds, where older, more independent siblings stay with their parents to help raising freshly hatched chicks or delay fledging until young siblings grow larger. While the effects of family life conflicts have been extensively studied, cooperation behaviours of siblings in subsocial species have long been neglected. Understanding the basis of sibling cooperation in these species could provide a key insight into the early evolution of social and family life. Here, we investigated sibling cooperation in the burying beetle *Nicrophorus vespilloides*, a species with facultative brood care, whereby offspring profits from the presence of parents but can also survive and develop in the absence of parental care. Recent studies suggest that siblings of this species cooperate in the absence of parental care to compensate for the parents' absence. The specific mechanism(s) of sibling cooperation in *N. vespilloides* are, however, unclear. To gain a deeper insight into the mechanisms involved, we tested whether older and therefore more independent siblings exhibit a greater level of cooperation than their younger counterparts. To this end, we compared growth and survival rates of newly hatched *N. vespilloides* larvae cohabitating with older larvae to those residing with age-matched siblings. Surprisingly, we found no effect of sibling age on either growth or survival rate. As the newly hatched larvae did not profit (but neither suffered) from the presence of more independent siblings, our first results suggest that the fitness effect of sibling interactions does not change with the age structure of a brood, but only depends on the number of siblings.

Smells like teen spirit: parented burying beetle larvae change their chemical profile during development

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Parental care often confers benefits to both caregivers and their offspring, but it also entails costs in terms of time, energy, and resources, leading to conflicts over care. To optimize resource allocation and enhance fitness outcomes for parents and offspring, it is beneficial to identify the needs of offspring at different developmental stages. Despite some examples, little is known about the cues or signals used to differentiate offspring developmental stages. Chemical signals and cues, which are commonly used by adult insects to communicate, are promising candidates. In this study, we aimed to investigate potential differences in the chemical profile of larvae at different developmental stages in *Nicrophorus vespilloides*, a species that is known to provide elaborate parental care. We used three distinct sampling methods to collect and analyze the cuticular lipids and the volatile organic compounds (VOCs) produced by larvae of all three instars. Our findings reveal quantitatively distinct cuticular and VOC profiles of the different instars. These differences might be used by parents to discriminate between larval stages and adjust their care behavior. Interestingly, we also found the volatile compound methyl geranate (MG) in the larval headspace samples. MG is a volatile pheromone produced by caring females in the presence of larvae to signal their temporary infertility to males in order to reduce mating attempts and coordinate parental care. We found that L2 larvae produce the highest levels of MG of all stages, consistent with the observation that L2 larvae receive the most parental care. Our data therefore suggest that MG might serve as a larval begging pheromone in *N. vespilloides*. Future studies should confirm this and further investigate the role of MG and other chemical compounds in parent-offspring interactions.

Recurrent genomic dynamics linked to parallel evolution of secondary phytophagy in Hymenoptera

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Repeated reversals towards secondary herbivory have occurred multiple times across the hymenopteran phylogeny and have been linked to increased rates of species diversification. This phenomenon indicates the significance of studying the genomic basis of dietary shifts in insects, as it can provide valuable insights into evolutionary innovation and adaptation mechanisms. In this project, we aim to investigate the genomic characteristics of specific groups within the order Hymenoptera, namely Aculeata and Chalcidoidea which descend from zoophagous ancestors but exhibit repeated reversals towards secondary phytophagy. Notable examples of such transitions are observed in gall-wasps and pollen-collecting bees, which have adopted a phytophagous lifestyle, while their closely related lineages have retained their zoophagous nature. This project builds on already available high-quality reference genomes and will be enhanced by newly sequenced genomes of species with high taxonomic relevance. To enhance our understanding of the evolutionary processes underlying the nutritional capabilities of Hymenoptera we use comparative genomics and transcriptomics to uncover genomic underpinnings of macroevolutionary dietary adaptations linked to e.g., the metabolism of plant secondary compounds, the composition of odorant receptors, gustatory receptor families, or carbon dioxide receptor genes. Further, we study genomic changes underlying evolutionary dietary shifts, testing the repeatability of gene gain and loss, and rapid evolution in regulatory sequences, transposable element dynamics, and gene copy numbers.

What Makes *Clunio*'s Circadian Clock Tick?

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Development and reproduction of the marine midge, *Clunio marinus*, are precisely timed by the circalunar and the circadian clock. The molecular components of both clocks have thus far not been studied. In Roscoff (Brittany, France), two sympatric *C. marinus* populations are found: one emerges only during the full moon, and the other only during the new moon. Previous analyses identified several mutations in the promoter of the period gene as the putative causal variants for the difference in lunar emergence in the two Roscoff populations.

The current project aims to test the effect of the mutations observed in the period promoter in vitro with a dual luciferase assay. Previous experiments have shown that transfection of *C. marinus* clock and cycle in S2 cells leads to luciferase activity driven by the artificial *Drosophila* period 3X69 promoter (Kaiser et al., 2016). We first want to identify which gene(s) repress(es) CLK and CYC activity in the *C. marinus* circadian clock. In the second step, we will replace the 3x69 promoter with the different versions of *C. marinus*' period promoter. This may elucidate the role of the period gene in both the circadian and circalunar clock.

On variation in phenotypic plasticity – what can we learn from the cosmopolitan freshwater snail model system *Physella acuta*?

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Phenotypic plasticity, which allows genotypes to express different phenotypes in different environments, is a highly relevant topic especially for adaptation and nature conservation. Recent meta-analyses find high variation in observed plasticity between the many studies that use different experimental approaches, individuals and populations. Unfortunately, we are only beginning to understand the reasons for such variation in plastic responses, which restricts our ability in extrapolating research findings on plasticity to natural ecosystems. Here, we report on new discoveries in this field that we made working with a classic plasticity model system, the freshwater snail *Physella acuta*. After introducing this species, I will first show that antibiotic treatment, which has been suggested to increase *P. acuta* survival under laboratory conditions, also impacts observable antipredator plasticity. Second, I will present first results on whether conspecific alarm cues, which are commonly applied to induce antipredator plasticity in this model system, retain their efficacy even after long-term storage at -20°C. While this method of storage is established in some antipredator plasticity model systems to minimize donor animal use, it is hitherto unexplored in gastropods. Our findings may contribute to a better understanding of the reasons underlying observable variation in phenotypic plasticity and enhance our predictive ability as to which studies' results can likely be generalized to the ecosphere.

Ultrastructure and fate of the protonephridia in the model organism *Platynereis dumerilii* (Annelida)

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Since long a pair of protonephridia is assumed to belong to ground pattern of the annelid trochophore. All trochophore larvae therefore should possess this kind of excretory organs. In a recent study, the existence of protonephridia in *Platynereis dumerilii* could not be shown. Since development of this species is fast and a high yolk content often hides these organs, we analyzed trochophore stages from the age of 20 hpf onward. The protonephridia appear at 24 hours and consist of a single terminal cell and a single duct cell. The terminal cell bears one cilium surrounded by a circle of 12 elongated microvilli and an outer, posteriorly directed, slashed cytoplasmic hollow cylinder (filter) of the terminal cell. The slits are bridged by diaphragms that represent the actual site of filtration. The duct cell is multiciliated and builds up the percellular duct. During development the microvilli and the filter are reinforced and the number of cilia in the duct increases. There is a right-left asymmetry in the entire developmental process, which ends at 70 hpf. At this point in time, the protonephridial duct cell detached from the terminal cell and is endocytosed. At this time the metanephridia of the following segment become functional.

Ultrastructural diversity of trichoid sensilla in a spider: did olfactory sensilla evolve from contact-chemoreceptive sensilla?

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Arthropods use cuticular structures to transduce specific environmental stimuli into a bioelectric signal. Among these, hair-equipped (trichoid) sensilla cover a variety of senses, such as mechano-, chemo-, thermo- and proprioception. To date, our knowledge of sensillar equipment and diversity in arthropods largely pertains to those on insect antennae. Among arachnids, most of what is known about sensilla concerns mechanoreception: there is a large body of evidence, especially for spiders, that sensitivity for touch, airborne and substrate vibration is highly developed. In contrast, olfactory capacities of spiders are poorly understood, especially with regard to which sensilla they use for odor detection.

Therefore, we investigated putative chemoreceptive sensilla on the walking legs of *Argiope bruennichi*, whose pheromone has been identified, synthesized and successfully tested in the field. Proximal podomeres of the walking legs of males are densely equipped with wall-pore sensilla known to be specialized for olfaction in insects. Ultrastructural features, such as the specific configuration of the sensory apparatus and surrounding sheath cells differ from those of insects and other arachnid taxa. Surprisingly, we did not find wall-pore sensilla on the legs of female *A. bruennichi*. Instead, we documented only numerous tip-pore sensilla, that also occur in males. Tip-pore sensilla are contact-chemoreceptors specialized in tasting liquid- or substrate-bound substances, but may also perceive volatile odors.

Our SEM- and TEM-study provides a comprehensive overview of the distribution and ultrastructure of trichoid sensilla present on the walking legs of *A. bruennichi*. We specifically focus on the (sub-)cellular organization of tip and wall-pore sensilla which we found to be similar in many aspects and different from those in insects. Therefore, it seems pertinent to assume that wall-pore sensilla of male *A. bruennichi* derived from tip-pore-like precursors. This implicates that wall-pore sensilla have evolved independently several times in arachnids and perhaps also in spiders.

The shape of water: adaptations of cochlea morphology in seals and otters

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Pinnipedia and Lutrinae are two related taxa in the group of Carnivora that returned to an aquatic lifestyle. Despite being the focus of numerous studies investigating their aquatic adaptations and ecology, how impedance matching in water and directional hearing work in those taxa is not completely understood. Using μ CT-scans, we analysed morphological traits of the inner ear and other auditory structures involved in sound perception in 38 taxa of the canine subgroup of Carnivora, Caniformia. To investigate the relationship between form and function we collected audiogram data for 20 (in air) or 9 (underwater) taxa respectively. The examination of cochlea shape space through principal component analysis showed that 82% of shape variation in our samples is explained by the first two principal components. Significant correlations for aquatic shape adaptations were found for numerous factors like: the number of cochlear turns, cochlea spiral height and width, and different shape types. From disc-shaped cochleae in seals to tower-shaped and pyramid-shaped cochleae in Mustelinae (weasels) and otters. Important anatomical traits not directly related to the cochlea include the area size of the fenestrae and tympanum, as well as the distance between tympanum and oval window. We identified changes in the proportions of the fenestrae and the tympanic sulcus and characteristic ratio differences for aquatic and terrestrial species. Additionally, we recorded enlarged openings of a bony canal from the round window to the bulla-mastoid junction. It is in close range to an opening in the skull base in all Pinnipedia, which conceivably relates to pressure compensation. This adaptation, the “external cochlear foramen”, has formerly only been known from Phocidae (true seals). Finally, our data suggest that numerous morphological changes in ears of aquatic taxa (reduced cochlear spiral height and number of turns, centroid size etc.) significantly affect hearing ability, notably the characteristic frequency.

On lips, tongues, and mouthparts: The cephalic fulcrum of insects reveals the true nature of the labium in remipede crustaceans

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Our current re-examination of the head morphology in remipede crustaceans by microtomography, histology, and scanning electron microscopy uncovered some major misinterpretations in previous anatomical studies. Among the flaws is the assumption that the so-called labium of remipedes is similarly formed by the second maxillae as in insects (Hexapoda). In remipedes, neither the skeleton, nor the skeleto-muscular system provides any evidence for an appendicular origin of the postoral lip forming the so-called labium. Instead, we detected paired sternal rods interconnecting the labium and the hypopharyngeal paragnaths with the paired intermaxillary apodemes. The rods correspond in position and shape to the hypopharyngeal fulturae of the entognathan apterygote hexapods (Collembola, Diplura). Functionally, the sternal rods and the fulturae also correspond in providing the point of articulation between the limb base of the first maxillae and the head. As in insects, the first maxillae of remipedes are jointed to the sternal rods by a separate skeletal element called the cardo that has been overseen in remipedes thus far. Detailed correspondences also concern the extrinsic muscles of the first maxillae and their basal endites that both in remipedes and insects display the same pattern with regard to their number and origin. We accordingly conclude that the sternal rods and fulturae in remipedes and hexapods identically act as a supportive fulcrum for the first maxillae. This correspondence unambiguously reveals the labium of remipedes as the homologue of the hypopharyngeal ‘tongue’ (lingua) of insects. The specific fulcrum-first maxillae nexus is unique among pancrustaceans and provides new support for the current view that Remipedia is the sister group of Hexapoda.

Morphometric Analysis of head structures in Mantophasmatodea

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The insect order Mantophasmatodea was described from two museum specimens collected many years ago in Namibia and Tanzania. In recent years, more than 20 additional species have been described from southern Namibia and the western and northern Cape provinces of South Africa. The always wingless species are often difficult to identify based on external morphology, although their genetic differentiation is usually clear. Klass et al. (Ent. Abh. 61: 3–67, 2003) used sclerites of the male post-abdomen to distinguish species, particularly those from the winter rainfall region of South Africa. These characters require dissection skills and are of limited use for non-invasive examination of museum specimens. We are currently investigating the morphological variability and taxon-specific differences in head shapes of males and females of different genera of Mantophasmatodea using CT scans. These scans were then evaluated with geometric morphometrics. First results show clear differences in the shape of the head capsule and mandibles, which might be related to differences in the prey spectrum. Our goal is to include all species of Mantophasmatodea, thereby providing sufficient information for an easy-to-use dichotomous key for species identification.

Applying nano CT-scan to the understanding and documentation of the skeletomuscular anatomy of a Cuckoo Bee (Apidae: Melectini)

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Bees encompass over 20,000 species, exhibiting diverse morphologies such as a disparity of body lengths (2 to 40 mm) and a wide array of behaviors, including brood cell nest parasitism, social organization, and specializations in pollen collection. Throughout the history of bee diversification, numerous phenotypic changes have occurred, including changes in their musculature and skeletal structures. In bees, the musculature is formed by approximately 200 muscle groups. This forms a complex system due to the intricate nature of some muscles and the challenges in deciphering their connections with the skeleton (referred to as skeletomuscular association). The knowledge about bee musculature primarily stems from studies conducted over 50 years ago, predating the advent of modern imaging techniques that have opened exciting possibilities for anatomical research. The objective of this project was to conduct a detailed investigation of the musculature and associated skeletal structures in a bee of the genus *Thyreus* (Apidae: Melectini). To achieve this goal, a 3D reconstruction based on nano-computed tomography was generated: a cutting-edge anatomical research technique. This approach allows for a comprehensive understanding of the skeletomuscular anatomy, which has been largely unexplored in bees from this perspective. Our results will contribute to the establishment of a solid knowledge of internal anatomy, and the high-quality documentation pursued in this research is unparalleled for bees. We expect that this work will enhance phylogenetic research and enable interpretations of adaptive and morphofunctional integration.

The eyestalk photophore of Northern krill *Meganyctiphanes norvegica* (Euphausiacea): innervation by irregular ommatidia of the compound eye

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The Euphausiacea are a taxon of holoplanktonic crustaceans the members of which are famous for generating bioluminescence using light organs (photophores). The specie's name *Meganyctiphanes norvegica* of the Northern krill studied here translates to "Norwegian bright shiner". These animals altogether feature ten ventral light organs, one pair of eyestalk photophores in the eyestalks, two pairs of thoracic photophores, and four unpaired pleon photophores. They generate light via a luciferin–luciferase type of biochemical reaction in light-emitting cells comprised in a photophore compartment called "lantern". Light intensity of all photophores can be modulated to fit the ambient situation and is under hormonal and neural control. The behavioural significance of bioluminescence in both Northern and Southern krill is discussed controversially. Exploring a possible function of light emission for communication between individuals, Fregin and Wiese (2002, *Helgoland Marine Research* 56:112–124) reported that exposing krill reared in a tank to artificial light flashes evoked a re-signalling behaviour, in which the animals pointed the beams of light from their photophores at conspecifics. In the current contribution, we provide a detailed description of the eyestalk photophore's histology. Furthermore, we report that the eyestalk photophore of *M. norvegica* is innervated by a distinct photophore nerve that originates from a specialized cluster of ca. 30 highly modified, "irregular" ommatidia at the dorsal rim of the compound eye that are optically isolated from the regular ommatidia. Our findings suggest the compound eye – photophore link as a major anatomical axis that may enable the animals to adjust their photophore activity to ambient light conditions and to perceive and respond to conspecific signaling. Acknowledgments: we wish to thank Christin Wittfoth, Vanessa Schendel, and the crew of the research vessel "Oscar von Sydow" of Kristinebergs Marina Forskningsstation, Fiskebäckskil, Gulmarfjord, Sweden for sampling *M. norvegica*.

Localization of tissues potentially involved in biosynthesis of ecdysteroids in the water bear *Hypsibius exemplaris* (Ecdysozoa, Tardigrada)

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Ecdysis or molting is a defining trait of Ecdysozoa, a group that includes arthropods, nematodes, priapulids, and allies. Especially arthropods require periodic molting for growth, as their body is covered with an inelastic exoskeleton, which has to be shed and replaced by a larger covering. The molt cycle is regulated by the ecdysteroid hormone ecdysone and its active form 20-hydroxyecdysone. These hormones are produced through stepwise hydroxylation from dietary cholesterol by a set of cytochrome P450 monooxygenases encoded by the so-called Halloween genes. While this process is well understood in insects and crustaceans, only little is known from other arthropods and their closest relatives, onychophorans (velvet worms) and tardigrades (water bears). Previous studies revealed that out of the seven Halloween genes present in the fruit fly *Drosophila melanogaster*, only shadow occurs in both onychophorans and tardigrades. To identify tissues potentially responsible for ecdysteroid biosynthesis in tardigrades, we analyzed the expression of shadow in the eutardigrade *Hypsibius exemplaris* using in situ hybridization. Our data show that shadow is expressed in the anterior part of the brain as well as the salivary glands in the head, and the claw glands associated with each leg. This indicates that ecdysteroid biosynthesis may occur in specialized tissues in all five body segments in tardigrades rather than being restricted to a single gland associated with either the prothoracic segment, as in insects, or maxillary segment, as in crustaceans. It would be interesting to know whether shadow is expressed along the body in onychophorans, as in tardigrades, or rather restricted to one of the cephalic segments, as in insects and crustaceans.

Evolutionary morphology of the visual system in cave-living spiders

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In caves, absence of light as a stimulus source and the compensation of vision by other senses are crucial for the evolution of specific morphological traits called troglomorphies. These adaptations include loss of pigmentation and the reduction of eyes. The composition of the visual system in Araneae (Chelicerata) is highly complex, but has only been studied in a few representatives so far. The principle eyes are composed of a chitinous lens, a cellular vitreous body, and an everted retina. Contrary, secondary eyes contain a retina of an inverted type and a tapetum. The visual pathways of each eye consist of two consecutive visual neuropils (lamina and medulla). The visual pathways of the principle eyes terminate in the arcuate body while the visual pathways of the secondary eyes converge in the mushroom body. Apart from this common pattern, different spider taxa show marked differences in their specific morphology, contributing to the disparity among spiders, which is externally manifested in the different positions and size ratios of the eyes.

The aim of the project presented here, which is in its initial phase, is to comprehensively examine the morphology of the visual system in different taxa of Araneae with epigean and troglobiont representatives to trace adaptations resulting from the multiple independent colonization of caves. Micro-CT analyses and immunohistochemical staining of histological sections will be used to examine the visual system in detail and provide the basis for subsequent morphological comparison. Evolutionary transformations will be discussed based on recent phylogeographical results elaborated with special attention to the colonization of the cave system in the Dinaric Alps. This region with its extensive cave-living araneofauna serves as model region to gain deeper insights into the convergent evolution of troglomorphic traits.

Self-sharpening mechanism in the mandibles of larval *Glossosoma boltoni* (Trichoptera, Insecta)

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Insect mouthparts interact with the ingesta (food or/and substrate) and show adaptations in shape, material composition and mechanical properties to it. Especially the foraging on hard ingesta, as on rocks covered by algae, is challenging since wear and structural failure can occur from the grazing activity. Adaptations to this are well studied in molluscs or sea urchins, but for insects there are large gaps in our knowledge. Here we focus on the mandibles of a grazing insect, the larva of the trichopteran *Glossosoma boltoni*. Using scanning electron microscopy, wear was documented on the mandibles. Its highest degree was identified on the medial surface of the sharp mandible tip. Using nanoindentation, the mechanical properties, such as hardness and Young's modulus of the medial and lateral mandible cuticles were characterised. We found, that the medial cuticle of the tip was significantly softer and more flexible than the lateral one. These findings strongly indicate that self-sharpening mechanism is present in this species.

To gain insight into the origins of the mechanical properties, we studied the degree of tanning using confocal laser scanning microscopy. The autofluorescence signal is strongly related to the mechanical property gradients. Additionally, we determined the content of transition and alkaline earth metals by energy dispersive X-ray spectroscopy. We found Ca, Cl, Cu, Fe, K, Mg, Mn, P, S, Si, and Zn in the cuticle, but the content was very low and did not correlate with the mechanical property values. These findings are not only valuable for biologists, but also for material scientists, as they contribute to our understanding of the origins of mechanical property heterogeneities in insect cuticle.

Input and output regions of the central complex of the cockroach *Rhyparobia maderae*

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Insects, like other animals, are able to navigate in diverse environments within their home range and beyond. To navigate safely, they integrate a variety of sensory inputs to adjust self-motion. The central complex (CX) in the insect brain acts as an interface between sensory input and motor control of navigational decisions (Pfeiffer 2023, Curr. Opin. Insect Sci. 55, 100972) and is, therefore, a brain area of high interest for diverse studies on navigational abilities of insects.

A particularly rich repertoire of navigational strategies has been investigated in cockroaches, including escape responses, obstacle negotiation, and path integration. To get a better understanding of the connectivity of the navigation network in the cockroach *R. maderae* we studied input and output areas of the CX. Following Neurobiotin tracer injections, single neurons were reconstructed morphologically to evaluate their ramification areas. Dendritic terminals were determined by fine fiber endings and axonal processes, by beaded/varicose specializations.

In most cases, tangential neurons, arborizing in particular layers of the CX, appear to be inputs, while columnar neurons with ramifications in single columns of the CX serve as outputs from the CX. Input regions of the cockroach CX include the bulb, the posterior slope (PS), the posterior optic tubercle, the inferior bridge, and the inferior clamp. The antennal mechanosensory and motor center (AMMC) and the wedge serve as output domains. Other brain regions are highly interconnected with the CX. These include the crepine, the lateral accessory lobe (LAL), the anterior lip, and the pedunculus of the mushroom body.

The data are consistent with the idea that visual and antennal mechanosensory information is integrated in the CX together with information signaling the behavioral context of the cockroach. Outputs could provide differential commands to right and left descending pathways for adapted goal directed steering.

A stochastic conductance-based model of the hawkmoth *Manduca sexta* olfactory receptor neuron

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The long trichoid sensillum in male hawkmoths, *Manduca sexta*, is innervated by two olfactory receptor neurons (ORNs) that respond to the pheromone released by female moths to attract conspecific mates. In the absence of odor stimuli, pheromone-sensitive ORNs in hawkmoths exhibit non-randomly distributed spontaneous spikes. These spikes occur within bursts and between bursts, and it is our goal to understand the interactions between the ion channels responsible for their generation. Therefore, analyzing spike distribution within and between bursts, as well as burst frequency and duration, is crucial for identifying different mechanisms at play. The random opening and closing of ion channels introduce internal fluctuations in neurons, known as channel noise, which contributes to the variability in spike distribution and determines whether a single spike or a burst occurs. Furthermore, insect ORNs serve as endogenous peripheral circadian clock neurons, leading to the expression of daytime-dependent rhythmic spike distributions.

In this study, we present a novel conductance-based model that incorporates the olfactory receptor coreceptor (ORCO) as a pacemaker ion channel with linear conductance dependent on cAMP concentration. Our model takes into account that cAMP express daytime-dependent rhythms with concentration being maximal during activity phase. By utilizing stochastic differential equations based on the microscopic Markovian states of ion channels, our model can reproduce the observed spike distribution with its circadian oscillations.

Knockdown of the molecular circadian clockwork in the Madeira cockroach *Rhyparobia maderae* deletes behavioural circadian rhythms

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Organisms evolved endogenous clocks that allow for anticipation of environmental rhythms such as the daily light-dark cycle. Endogenous clocks are based upon positive feedforward and negative feedback loops orchestrating timing of rhythms in physiology and behaviour. The molecular clockwork of circadian clock neurons controlling sleep-wake cycles is studied best in the insect *Drosophila melanogaster*. It is based upon transcriptional-translational feedback loops (TTFLs) of clock genes. The transcription factors Clock (CLK), or Cycle (CYC) are positive feedforward elements activating transcription of the negative feedback elements Period (PER) and Timeless 1 (TIM1) that suppress their own transcription. Circadian TTFL clockworks of mammals and some insects such as the Madeira cockroach *Rhyparobia maderae* employ Cryptochrome 2 (CRY2) as another negative feedback element. In the Madeira cockroach transplantations identified the brain's accessory medulla (AME) as clock network controlling sleep-wake cycles. Its circadian neurons' clockwork is only partly known, with the negative feedback elements PER, TIM1, and CRY2. So far, RNA interference (RNAi) induced individual knockdown of all known negative feedback elements did not delete circadian sleep-wake rhythms. Furthermore, modelling studies suggested that there is an additional negative feedback element missing and that TTFL clockworks differ in distinct AME clock neurons. Since in mammals only deletion of CYC caused behavioural arrhythmicity, we first identified the positive feedforward elements CLK and CYC, before attempting to stop the TTFL clockwork with RNAi. Here, we identified the genes *clk* and *cyc* of the positive feedforward loop of the TTFL clockwork in the Madeira cockroach. Furthermore, via RNAi induced single and double knockdowns of their respective mRNAs we succeeded to disrupt circadian sleep-wake rhythms in *R. maderae*. Future genomic analysis and, also transcriptomic studies at the single cell level will reveal the structure and function of molecular clockworks in individual clock neurons of the AME clock.

Tuning to the rhythms: pheromone-sensitive olfactory receptor neurons of the hawkmoth *Manduca sexta*

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Sensory neurons are tuned to detect and predict various environmental rhythms as an elementary advantage for the animal's survival. We examine and model the molecular mechanisms that allow pheromone-sensitive olfactory receptor neurons (ORNs) of the nocturnal hawkmoth *Manduca sexta* to entrain to oscillations in pheromone presence at circadian and ultradian time scales. For mating, female hawkmoths release pheromone pulses at ultradian frequencies during the night to attract their conspecific mates. The frequency of pheromone pulses encodes the female's location. Thus, for successful reproduction males and females need be active at the same time of day. Insect ORNs are endogenous circadian clock neurons that comprise a circadian clockwork in the nucleus based on transcriptional translational feedback loops (TTFLs). These clocks regulate daily sleep wake cycles and allow for synchronization of male and female behavior. However, the male's pheromone sensitive ORNs are additionally required to resolve ultradian frequencies of pheromone signals for female location. It is not known whether/how the TTFL-clock controls circadian and ultradian rhythms in sensitivity and temporal resolution of ORNs during the day.

We used in-vivo electrophysiological (tip-) recordings from long trichoid sensillae of male hawkmoths to characterize the two pheromone-sensitive ORNs. Rhythms in spontaneous activity with spikes differently distributed within or between bursts indicated at least two ultradian frequencies of membrane potential oscillations. It is not known whether both ultradian membrane potential rhythms are linked and are controlled via the circadian TTFL clockwork in ORNs.

We found that spontaneous spike activity of ORNs expressed interlinked rhythmicity in circadian and both ultradian frequency ranges. Subsequently, we employed pharmacology and computational modelling to examine the role of the HCN-type pacemaker channel and of ORCO, the olfactory receptor coreceptor for control of spontaneous activity. With these studies we attempt to decipher the ORNs' temporal encoding.

Localization of pigment-dispersing factor neuropeptides reveals conserved and derived patterns across panarthropods

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Pigment-dispersing factors (PDF) are a conserved family of neuropeptides that occur in several protostome groups, including the lophotrochozoan and ecdysozoan lineages. First reported as pigment-dispersing hormones (PDH) from decapod crustaceans, these peptides have been intensely studied in crustaceans and insects. While being responsible for integumental color change and dispersion of shading pigments in the eyes of various crustaceans, PDF additionally acts as an output factor of the circadian clock in many insects and possibly also crustaceans. Comparative studies focused mainly on the distribution of pdf genes across Panarthropoda, which includes Tardigrada (water bears), Onychophora (velvet worms), and Arthropoda (spiders, centipedes, crustaceans, insects and alike). Two pdf homologs have been identified in onychophorans, which were most likely inherited from the last common ancestor of Ecdysozoa (the clade of molting animals). Notably, one of these homologs, pdf-II, was lost either in the arthropod or arthropod/tardigrade lineage, followed by multiple duplications of the remaining pdf-I gene in tardigrades and some crustaceans. Besides onychophorans, tardigrades and crustaceans, all other panarthropod taxa, including chelicerates, myriapods and insects, possess only one pdf gene, which is the homolog of pdf-I. Across panarthropods, PDF expression was only studied in insects and some crustaceans and onychophorans, while the remaining taxa have been neglected. We therefore performed immunohistochemical labeling of putative PDF-immunoreactive cells using specific and cross-reactive antibodies in tardigrades, spiders, centipedes, and annelids as an outgroup. Our data suggest extensive reduction of PDF-expressing cells either independently in tardigrades and arthropods or in the arthropod/tardigrade lineage, depending on the phylogenetic position of tardigrades. Our findings further provide insights into the expression pattern and potential function of PDF neuropeptides in chelicerates and myriapods.



Hopper by name, hopper by nature. Decision-making processes underlying the locust startle response

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Swarms of the migratory desert locust can extend over several hundred square kilometers, devouring everything in their path. Despite their immense socio-economic impact, little is known about the mechanisms underlying their collective decision-making processes. Therefore, we combined behavioral experiments of freely walking animals with fine-scaled recordings of descending neurons to shed light on these processes.

Navigating complex environments while avoiding threats is central to a locust's survival. In order to make an informed escape decision, animals can utilize both individually acquired (e.g., the looming silhouette of a predator) and socially derived information (e.g., startle response of conspecifics). Here, integrating and weighing different information streams is crucial for effective escape responses while conserving valuable energy resources. In our study, we investigate the initiation of individual startle responses and their propagation in groups of gregarious desert locusts to unravel the underlying decision-making processes. Controlled lab experiments with single, freely moving animals provide insights into the characteristics influencing an individual's response, revealing a non-linear relationship between stimulus intensity and response probability. Combining these stimulus-response characteristics with recordings from descending motion detection neurons can give insight into neuronal evidence accumulation processes. By employing a virtual environment, we can investigate how the startle response of virtual locusts impacts the decision of a single animal, and how the combination of social and environmental stimuli affects the stimulus-response characteristic. All in all, we aim to shine a new light on the principles underlying the orthopteran escape decision by employing an overarching, naturalistic approach that can give insight into the collective decision-making processes of a harmful pest species.

Navigating the Three-Dimensional World: Challenges and Strategies of Bumblebees

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Navigation is crucial for all animals regardless of their locomotory style, whether walking, swimming or flying. Although walking animals experience changes in altitude, the issue of 3D navigation becomes even more apparent for swimming or flying animals such as fish, bats, birds, or bees. Bumblebees are faced with 3D challenges when they need to find their nest and travel towards flowers. Their nest is below the ground in a cluttered terrain where the visual scenery will change drastically outside and within the clutter.

Pinpointing a location from a high altitude, like the top of a mountain, might be an easy task. However, as soon as one enters the valley, the bird's eye views might not be useful for pinpointing a location. Views closer to the ground with more occlusions of nearby objects might be more challenging. We could show that bees return within the artificial clutter to their nest, although classical view-based models predicted a return above the vegetation.

Bees use local cues while targeting goal locations on the floor in 2D. However, natural food sources collected by bees, such as flowers of a tree are largely spread in 3D with height differences of multiple meters. Manipulating the feeder position and surrounding cues on a few meters range revealed that bumblebees could learn a feeder's height only when it is close to a global reference, e.g. the floor, and a local reference.

Identification of core hub genes via transcriptome sequencing analysis of clock-controlled pheromone transduction in *Manduca sexta*

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Insect pheromone transduction is under strict circadian clock control. We study pheromone transduction and its regulation via endogenous clocks in the hawkmoth *Manduca sexta*. While it is generally assumed that insect olfactory receptor neurons (ORNs) employ an odor receptor (OR)-coreceptor (Orco) receptor-ion channel complex for pheromone and general odor transduction, in the hawkmoth we find no evidence for ionotropic pheromone transduction. Instead, with in vitro and in vivo electrophysiological recordings of nocturnal hawkmoth ORNs we find evidence for a G-protein coupled pheromone-transduction cascade involving phospholipase C activation. Furthermore, we find daytime-dependent modulation of the transduction cascade controlled via endogenous clocks, such as circadian clocks in ORNs.

Since the molecular mechanism and members of insect pheromone/odor transduction and its circadian modulation are unclear, here, we investigated dynamic transcriptomic changes in adult male hawkmoth antennae at different zeitgeber times (ZTs) comparing sleep- and activity phases, in search for core hub genes of clock-regulated pheromone transduction. Preliminary studies identified an array of potential participants in pheromone transduction, encompassing 7 G-proteins, 123 ion channels, 43 participants of second messenger cascades, and 90 enzymes that potentially participate in pheromone transduction, which are implicated as candidate genes, and transcript variants. Furthermore, the circadian clock including period, timeless, clock, cycle, cryptochrome, and other 11 candidate genes, exhibited significant differences across different ZTs, suggesting circadian clock-dependent regulation of pheromone transduction. Subsequently, we will perform functional assays to challenge the role of these candidate genes in pheromone transduction, utilizing Ca²⁺ imaging, patch clamp, pharmacology, CRISPR/Cas, and RNA interference (RNAi), also in cockroach antennae.

In summary, this study elucidates the core hub genes involved in the clock-regulated pheromone transduction in *M. sexta*, contributing to our understanding of the molecular mechanisms and physiological regulatory network underlying insect olfactory perception.

Circadian neuropeptidomics for the analysis of coupling factors controlling multiscale behavioral rhythms in *Drosophila melanogaster*

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Circadian rhythms allow animals to synchronize behavioral and physiological processes to the environment. These rhythms are produced by core clock neurons in the nervous system that generate and transmit time-of-day signals to downstream tissues, driving overt rhythms. These cells drive and time the major activity peaks at dusk and dawn. The fruit fly *Drosophila melanogaster* uses the active phase, for example, for feeding. This behavior is regulated by signalling molecules such as neuropeptides. To identify potential peptidergic candidates involved in the regulation of feeding behavior at dusk and dawn, we used MALDI-TOF mass spectrometry (MS) and Q-Exactive Orbitrap MS to investigate the circadian neuropeptidome of L3 *Drosophila* larvae under fed and starved conditions. To identify potential neuropeptides regulating feeding behavior and overall activity at different times of the day, flies were treated under fed and starved conditions (24 and 48 hours). Their activities were recorded for 20 minutes before the end of the starvation period, respectively. Statistical comparison of the resulting mass spectra revealed semi-qualitative changes in the ion signal intensities to products of eight neuropeptide genes (sifamide, sNPF, extended fmrfamide, hugin-pk, calcitonin-like diuretic hormone-31, allatostatin-C, corticotropin releasing factor-like diuretic hormone-44, kinin). Behavioral analysis showed differences between the fed and the starved conditions, depending on the time of the day. Based on the semi-quantified results, we started with single cell analysis by MALDI TOF MS using different gfp-GAL4 constructs for cell identification and manipulation. The results of our study provide necessary input for future measurements up to single cell level to study the dynamics in up- and downregulation of neuropeptides underlying mechanisms regulating feeding.

The circadian clock in lower dipterans – An anatomical study

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Nematocera as lower dipterans comprises several superfamilies within the order Diptera. They inhabit many ecological niches and even switch between niches during their development, therefore they experience a variety of daily and seasonal environmental changes. Nematocera spend most of their life as larvae, while the imagines are short living and show mostly a highly reduced food intake. The lower dipterans have ecological importance as pollinators, and disease vectors and play a major role in the food chain and as decomposers of organic matter. As part of the dipterans, they are closely related to the model organism *Drosophila melanogaster* but share characteristics of a more ancestral molecular circadian clock found in other insects such as Lepidoptera. As most studies in Nematocera focus on mosquitoes as disease vectors, less is known about the more general organization of the circadian clock across the lower dipterans.

We use immunohistochemical approaches to characterize the neuroanatomy of the circadian clock in several members of the different superfamilies (Bibionomorpha, Psychodomorpha, Culicomorpha) with a main focus on *Clogmia albipunctata*. By staining with antibodies against the *Drosophila* clock protein PERIOD we demonstrate that lower dipterans have a *Drosophila*-like organized clock network with lateral and dorsal cell clusters and daily oscillating PERIOD levels. Furthermore, we stained against the neuropeptide Pigment Dispersing Factor (PDF), the main output factor of the circadian clock in other insects and found several PDF immunoreactive cell clusters close to the accessory medulla, which colocalize with anti-PERIOD staining. PDF-positive fibers innervate the optic lobes and the dorsal protocerebrum but in contrast to *Drosophila*, contralateral projections are virtually absent.

We seek to develop a general understanding of the clock network organization in Nematocera that could serve as a basis for further studies focused on the control of rhythmic behaviors in lower dipterans.

Temperature-dependence on neuropeptide titers of individual *Drosophila* Corazonin-expressing neurons in the CNS by quantitative immunocytochemistry

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Neuroactive substances like 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 neurons of larval and adult fruit fly *Drosophila melanogaster* by quantitative immunocytochemistry. Corazonin is 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 performed whole-mount immunofluorescence stainings against corazonin (Crz) on L3 larval CNSs and adult CNS. Statistical comparison revealed higher signal intensities of Crz-ir in both larvae 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 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.

Neuronal and molecular characterization of the circadian clock network in a polar pelagic key species – the Antarctic krill (*Euphausia superba*)

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Biological clocks are based on widespread, conserved, molecular mechanisms which allow organisms to anticipate regular daily and seasonal changes in their environment and adapt their physiology and behavior accordingly. The Antarctic krill (*Euphausia superba*, hereafter krill) is a key species, endemic to the Southern Ocean ecosystem and a species with one of the highest biomasses on earth. Being the major food source for apex predators such as whales, penguins, and seabirds, krill is channeling energy from the base of the food web directly into the highest trophic levels and is thus of disproportional importance for ecosystem functioning in the Southern Ocean. Krill shows both daily and seasonal rhythmicity in gene expression, physiology, and behavior, which is synchronized with the extreme photic conditions in its high-latitude habitat.

In this study, we highlight the neuroanatomy of the krill's central brain and optic lobes, regions that are known to be involved in controlling important physiological and behavioral functions and, in other species, to host the main pacemaker of the circadian clock. Using antibodies directed against insect synaptic protein Bruchpilot (nc82), we identify the optic lobe neuropils (lamina, medulla, lobula, lateral protocerebrum) as known from decapod crustaceans and insects. In krill, the optic lobe neuropil structures are connected by two optic chiasmata which convey visual information from the retina to the central brain. Further, staining against the crustacean neuropeptide Pigment Dispersion Hormone (PDH) reveal clusters of PDH-positive neurons and their arborizations connecting areas in the central brain with neuropil structures in the optic lobe. Further, we currently attempt to locate neurons expressing key clock genes as period, cryptochrome 2, and timeless using in-situ hybridization. Combining these analysis with temporal clock gene expression data from qPCR we aim to reveal the structure and characteristics of the circadian neuronal network in this ecologically important species.

Localization of multiple rhabdomeric opsins in the water bear *Hypsibius exemplaris* (Eutardigrada)

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Tardigrades (water bears) are the closest relatives of arthropods (insects and allies) and onychophorans (velvet worms), with which they are united in Panarthropoda within the clade of molting animals (Ecdysozoa). Due to their phylogenetic position and unique abilities to survive extreme environmental conditions, tardigrades play an important role for understanding animal evolution and function of cells and tissues. This includes the spatial distribution of opsins – light-sensitive, membrane-bound proteins – of which nine have been detected in the “model” tardigrade *Hypsibius exemplaris*: four rhabdomeric opsins (r-opsins), three ciliary opsins (c-psins), and two neuropsins. By analyzing mRNA sequence reads from *H. exemplaris* and another eutardigrade, a recent study revealed ontogeny-related differences in the expression of opsins. However, beyond this nothing is known about the expression and spatial distribution of opsins in tardigrades. We therefore performed HCR (hybridization chain reaction) in situ hybridization to localize all four rhabdomeric opsins in *H. exemplaris*. Our mRNA expression data revealed that while one of the r-opsin genes (r-opsin-v) is expressed in the rhabdomeric cell of the eye the other three r-opsin genes (r-opsin-1, r-opsin-2, and r-opsin-3) are co-expressed in a cluster of cells, which is adjacent to the rhabdomeric cell within the outer lobe of the brain. These results indicate that r-opsins might have multiple functions, one associated with vision and others with other physiological processes, such as circadian photoentrainment and thermosensation.

Impact of different enriched rearing regimes on the neuroplasticity in the brain of marbled crayfish *Procambarus virginalis*

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The parthenogenetic marbled crayfish that produces exclusively genetically identical offspring exhibits high phenotypic variability despite its lack of genetic variation making it an excellent model organism for studying neuroplasticity. For investigating environmental-induced changes in the nervous system of marbled crayfish, 35 individuals of same age from one mother were separated into impoverished and enriched rearing regimes. After being exposed to the different rearing conditions for different lengths of time, each animal was tested and compared for their allometric growth and synaptic plasticity in the hemiellipsoid body, a high-order multisensory center which is presumed to be responsible for learning and memory. A correlative microscopy approach was taken. This included x-ray microscopy to obtain brain volumes and immunohistochemistry with confocal laser scanning microscopy. By using the animals' cephalothorax lengths and the 3D-reconstructions from the microCT-scanning as references controlling for allometric growth, volumetric changes of the total brain and the hemiellipsoid body of individuals of both rearing regimes were compared. Synaptic densities in the hemiellipsoid body were compared as revealed by immunohistochemical labeling against synapsin (presynaptic contacts) and glutamic acid decarboxylase (GAD) (postsynaptic contacts of mainly inhibitory neurons). Separation itself seemed to result in an initial growth spurt of the hemiellipsoid body in both rearing regimes. Mean total brain volumes of individuals under enriched conditions appeared to stay more constant whereas they fluctuated under impoverished conditions. The amount of GABAergic synapses as well as the synapsin-immunoreactive synapses were slightly higher over time under impoverished conditions than under enriched conditions. Although the findings of our pioneer-study do not allow general conclusions to be drawn for the neuroplasticity of marbled crayfish, our findings suggest changes occur fast and are highly dynamic.

Validation of RNAi mediated IR25a chemoreceptor protein knock-down in *Daphnia longicephala* using immunohistochemistry

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Phenotypic plasticity is crucial for animals to enhance their fitness in changing environments. For example, the emergence of predators induces the development of morphological defences in the freshwater crustacean *Daphnia*. These defences make *Daphnia* less susceptible to predation and thereby their fitness is increased. Inducible defences are developed upon the perception of predator-specific chemical cues. The involved chemoreceptors are encoded by a gene family belonging to the group of ionotropic receptor proteins that evolved from ionotropic glutamate receptors. RNA interference (RNAi) mediated knock-down of the co-receptor genes IR25a and IR93a made *Daphnia* unresponsive to predator-specific chemical cues. However, validation of gene knock-down was only validated through quantitative PCR and was thus limited to the RNA level.

To study the effects on the protein level, we designed and obtained a custom-made polyclonal antibody for the *Daphnia* IR25a co-receptor. Using immunohistochemistry in line with confocal imaging, we show that IR25a proteins are located on the cell membrane of the olfactory sensory neurons located in the vicinity of the aesthetascs of *Daphnia longicephala*. With the help of intensity measurements of secondary fluorescent antibody staining of the OSN as the region of interest, and with respect to a reference region, we document the significant reduction of the overall intensity in RNAi microinjected specimen. Based on this we conclude that the gene knock-down leads to the reduction or even loss of the IR25a protein. Specimens where the IR25a gene was knocked down and the IR25a protein is absent do not develop defensive features when exposed to predators i.e. *Notonecta spec.* Our study provides the first evidence that IR25a proteins are indeed located on the chemosensory antennules and the knockdown of the IR25a protein is responsible for the loss of chemosensory perception of predators.

PyView: A general purpose tool for analyzing calcium imaging data

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Optical imaging allows to record network activity in many neurons simultaneously, yielding a direct access to network activity in the brain. The development of transgenic animals that express genetically encoded calcium reporters, such as GCaMP, has further increased the possibilities to study neural networks, other reporters, suitable for measuring membrane potential or specific second messengers are being developed in several labs. All of these techniques use changes in fluorescent light to quantitatively capture changes in neuron physiology, and all necessitate high-level image analysis tools in order to analyze the physiological data in a quantitative and consistent way.

Here, we propose a software for optical imaging analysis: pyVIEW (<https://github.com/galizia-lab/pyview>). pyVIEW splits data treatment into two steps: a powerful GUI for interactive data analysis, with flexible selection of evaluation parameters, and a second step for batch processing, yielding time traces, 2D images or 3D movies across all experimental measurements with identical parameter settings. The program is modular, and easy to expand with tools for dedicated analyses and/or experimental questions. The program is open source, written in python, and can be expanded to act as a wrapper for other programs that address single steps in a data analysis pipeline.

Information about example workflows, galleries of examples outputs, and guides for installing, using and developing pyVIEW are organized in a wiki (<https://github.com/galizia-lab/pyview/wiki>)

Distribution of identified orcokinins and partially co-localised orcomyotropin in central and peripheral neurons and neurosecretory cells of the crayfish *Faxinus (Orconectes) limosus*.

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Discovered as hindgut-bioactive peptides in the spiny cheek crayfish *Faxinus (=Orconectes) limosus*, the tridecapeptides orcokinins (OK) and orcomyotropin (OMT, FDAFTTGfamide) are now identified almost all arthropods. We produced rabbit antibodies to Asn13-orcokinin (Asn-OK) and OMT, and by conventional cloning and mass spectrometry, we partially analysed the identity and distribution within the central and peripheral nervous system (CNS, PNS). We found OKs to be much more abundant than OMT in the entire CNS and only partially co-localised even that both apparently arise from the same precursor molecules. OKs occur almost exclusively in all primary olfactory sensory neurons (OSNs) of the outer flagellum aesthetascs of the first antennae (Ant1) projecting directly into the olfactory lobe (OL) of the brain, whereas there are no OMT-immunoreactive (ir) neurons in the brain. We have cloned two mRNA-precursors for OKs from Ant1-s and the brain encoding 7 copies of Asn13-OK and single copies of three OK-isoforms and de novo-sequenced the main peptide Asn13-OK from Ant1-extracts by mass spectrometry. Numerous OK-ir local interneurons are associated with all neuropils in the eyestalk, brain and ventral nerve cord. Associated with the suboesophageal ganglia and peripheral pericardial organs, there are several typical neurosecretory neurons. A prominent neuron group in the terminal abdominal ganglia containing co-localised OKs and OMT forms terminals on the entire hindgut muscles. Further distinct OK-ir neurons occur in the stomatogastric nervous system. Thus, the wide-spread OKs and OMT are important elements of sensory and interneuronal information processing and myoactive modulation in crayfish.

From a naive bee to an experienced forager: How different guiding mechanisms direct bees through cluttered environments

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Bumblebees are excellent navigators and have been observed to travel along routes and optimize them between multiple feeding locations. When foraging, obstacles often require the bees to deviate from their path to avoid collisions. However, it is still unknown how bees establish these routes and how experience affects their flight paths in clutter. By recording flights of novice bees, which had never flown in cluttered terrains, as well as experienced bees, we show that bees quickly learn to cross the clutter efficiently. With increasing experience, bees need less time to cross the clutter and their flight paths become straighter. Bees display a behaviour that could be explained by an interaction of different mechanisms, such as following the most familiar scenery, avoiding obstacles, and aiming for their nest along their routes. We studied the underlying mechanisms by comparing model responses to the flight paths of bees with different levels of experience in clutter, using a causality detection method (convergent cross-mapping). Our findings indicate that experienced bees are primarily guided by a global direction cue, rather than following a route based on image familiarity. Our results shed light on the route-following mechanisms that drive bees at different stages of their foraging life.

Dynamic visual control of flight at low light levels in the nocturnal hawkmoth *Deilephila elpenor*

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Flying insects use visual information to control and stabilize their flight. In nature, this information is highly dynamic, presenting a challenge for insect brains to extract reliable and relevant information from it. And yet, many animals master these challenges on a daily—and nightly—basis. To understand this ability, it is not sufficient to consider the sensory processing of natural stimuli by itself. Instead, behaviour and sensory processing act in concert: as senses guide an animal's movements, the movements in turn shape the sensory input. Thus, the contribution of locomotion to perceiving sensory information must be considered. For example, when driving faster, the world moves past our eyes faster, shifting visual input to higher temporal frequencies. Similarly for flying insects, alterations in flight speed and distance to objects shape the spatiotemporal frequency composition of their visual input. Thus, flying animals can shape their movements to support optimal visual acquisition – this can be particularly important in challenging light environments - in very dim light, or when light levels change suddenly. Here, using the nocturnal hawkmoth *Deilephila elpenor*, we studied how flight features (e.g. speed, straightness, etc.) are dynamically adjusted at different natural light intensities to aid visual acquisition. We recorded moths' flights in a lab-environment that is large enough to allow the animals to choose their flight strategies flexibly and use their full repertoire of flight manoeuvres. Moreover, ambient light intensities were matched to three natural conditions within the moth's visual activity range (starlight, moonlight, and twilight). We discuss how the visual environment impacts the animals' flight performance, measured as translational and rotational velocity, flight height off the ground, distance to surrounding structures, as well as straightness of their paths.

Challenges and approaches for measuring whole-brain activity in non-model insects

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The insect world constitutes of an enormous variety of behavioral repertoires across innumerable species. This comprehensive dictionary offers an excellent opportunity to understand how nervous systems generate algorithms for solving reoccurring questions. Despite these opportunities in the field of evolution and neurobiology, exploring underlying neuronal correlates is challenging due to the lack of specific tools for most of these species. Existing tools have drawbacks. Importing genetically encoded calcium markers is arduous and costly, even with the availability of the CRISPR-Cas9 system. Calcium and voltage dyes can be lacking in photostability. Electrophysiology requires extensive training and can be limited in spatial information. Based on rapid gene expression, immediate early genes have been used, especially in mammalian systems, to characterize recent behavioral history. Such tools can offer a window to unbiased, hypothesis-free, exploratory whole-brain analyses without focusing on a particular brain region. Here we are leveraging our expertise in peripheral sensory modalities in insect organisms. Using controlled olfactory stimulus delivery, we explored odor-driven responses in the desert locust, *Schistocerca gregaria*, and other insect species. We utilized straightforward processing pipelines to create species-specific template brains onto which activity maps could be registered. We hope this package will expand the number of species investigated in the insect neurobiology.

Processing of wide-field motion in the central brain of bumblebees

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Moving animals experience wide-field motion through the displacement of the retinal image during motion. This provides information about rotational 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.*). While in honeybees and bumblebees the use of wide field motion for behavioural tasks is well investigated, little is known about the processing of wide-field motion at the neuronal level. Here we investigated tuning properties of motion-sensitive neurons in the central brain of bumblebees. To do so we conducted extracellular tetrode recordings from neurons responding to bilaterally presented wide-field visual front-to-back or back-to-front gratings with different temporal and spatial frequencies. A first classification of the resulting units is based on the spike count during stimulation relative to the background activity level. Most units responded excitatory ($N = 82$, $n = 45$) or inhibitory ($N = 5$, $n = 5$) to front-to-back and back-to-front motion. Other units showed direction selective responses. They either showed inhibitory ($N = 7$, $n = 7$) or excitatory ($N = 25$, $n = 20$) responses only to one of the stimulus directions or antagonistic responses ($N = 17$, $n = 15$) where one stimulus direction lead to excitatory responses and the opposite direction lead to inhibitory responses. Taken together, the tuning among all described groups implies differences in selectivity for different functionalities, mainly for direction and spatiotemporal frequency. Considering group-specific features combined with receptive field properties, and anatomical information allows to link individual groups to behavioural functions.

The plasma membrane as multiscale posttranslational feedback loop oscillator

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The ability to predict environmental changes such as daily light-dark cycles increases the chances for organisms' survival. Thus, endogenous clocks, such as circadian clocks that anticipate the 24h light-dark cycles, evolved to translate the external environmental rhythms into an intracellular response. Two different types of clocks can be distinguished: transcriptional translational feedback loop (TTFL)- and post transcriptional feedback loop (PTFL)-clocks. They generate oscillatory outputs that couple a plethora of multiscale clocks throughout the body via unknown mechanisms.

In insect peripheral and central clock neurons we study components and mechanisms of plasma membrane associated multiscale oscillations, such as oscillations in membrane potential, intracellular Ca^{2+} , and cyclic nucleotide levels, searching for multiscale interactions. Both central peptidergic clock neurons of the Madeira cockroach and hawkmoth olfactory receptor neurons, which are peripheral circadian clocks, express circadian and ultradian membrane potential oscillations via unknown mechanisms. While patch clamp and extracellular recordings of primary cell cultures of neurons are established, and routine recordings of intracellular Ca^{2+} levels are performed with calcium indicator dyes such as Fura-2, we establish parallel FRET recordings with biosensors like Epac-SH187 and cGi-500 to measure cyclic nucleotide level changes in vivo and in real-time.

We predict that ultradian and circadian membrane potential oscillations are generated via pacemaker channels together with antagonistic channels constituting a PTFL clock. This membrane clock generates potential oscillations accompanied by intracellular Ca^{2+} oscillations via voltage-dependent Ca^{2+} channel activation. Cycling intracellular Ca^{2+} levels are hypothesized to drive cAMP production periodically via Ca^{2+} /calmodulin-dependent adenylyl cyclase, activating membrane-anchored protein kinase A. With various biochemical assays we examine spatiotemporal regulation and crosstalk of Ca^{2+} and cAMP signaling cascades on the circadian and ultradian scale in biological clocks and challenge our hypothesis of the plasma membrane as an endogenous PTFL clock that is coupled to, but not driven by, the TTFL nuclear clockwork.

Geographical variation in locomotor activity rhythm and cycling of clock proteins at the brain level in *Drosophila littoralis*

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Drosophila littoralis is a fly species belonging to the virilis group, widely distributed in Europe and found in latitudes between 41°N and 69°N. Interestingly, the northernmost populations live in arctic regions and face harsh winters and extremely changing day lengths throughout the year. To adapt to these conditions, they developed a strong light-dependent overwintering response (called diapause). Conversely, southern strains, which do not face extreme seasonal changes, show a weaker diapause. Given the importance of light in the overwintering response of northern strains, a long-lasting question is whether the circadian clock, which times daily behavior and is a reference timer to measure day length, shows adaptations to changes in day length. Previous studies suggested that the strength of circadian clocks weakens with increasing latitudes and that weak clocks synchronize better with changing day lengths. Instead, the rhythmic clocks of southern species seem less flexible. For *D. littoralis*, this rule seems to apply within the same species: eclosion rhythms strength decreases with increasing latitudes.

To assess whether the circadian clock shows a latitudinal adaptation, we studied the circadian locomotor activity of *D. littoralis* and measured their rhythmicity in constant darkness, as expected, the southern strains showed a stronger rhythmicity. To evaluate the strength of their molecular clock within the brain, we measured the intensity of PAR-domain clock protein 1 (PDP1) staining, which cycled in the southern strain but not in the northern one. Since the rhythmic release in the brain of the neuropeptide Pigment Dispersing Factor (PDF) is essential for maintaining the activity rhythm in *D. melanogaster*, we measured the staining intensity of PDF and showed that it cycled in the southern strain. Finally, the northern strains showed weaker clocks, which can adapt to the northern extreme light conditions, whereas the southern strains lacked this characteristic and maintained stronger circadian clocks.

Early development of the primary olfactory centres and their neurochemistry in two malacostracan crustaceans with different life histories – a comparative approach

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The crustacean central olfactory pathway is a complex system with two primary olfactory centres in the deutocerebrum, the so-called olfactory lobes. The fundamental functional differences between the olfactory pathways of malacostracan crustaceans and Hexapoda are a topic of ongoing discussion. However, less information is available on the development of the central olfactory pathway in Malacostraca compared to Hexapoda.

We used immunohistochemistry to analyse when different anatomical and chemical properties of the olfactory pathway emerge during the early development of two malacostracan species with different developmental modes to gain insight into their functions.

Parhyale hawaiiensis (Amphipoda, Peracarida) develops directly. Hatchlings have almost the same benthic lifestyle and sensory environment as adults. Contrary, *Hemigrapsus sanguineus* (Brachyura, Decapoda), undergoes metamorphic development with five planktonic zoeal stages, a semi-benthic megalopa stage and a benthic adult. These lifestyles likely differ in their sensory requirements. We studied embryonic and early post-embryonic stages of *P. hawaiiensis* and zoeal stages I and II of *H. sanguineus*.

Immunostaining against Synapsin is commonly used to image the analytical subunits of the olfactory lobes, the so-called glomeruli. Allatostatin staining is known to show the subdivision into cap- and base-structures in the glomeruli. Our results show developmental differences between the two species in Allatostatin and Serotonin immunoreactivity, and in the shape of the glomeruli as seen in Synapsin stainings. In *H. sanguineus*, these aspects differ markedly between larval stages and adults, while in *P. hawaiiensis*, differences are minor. Conclusion: The primary olfactory centres of *H. sanguineus* larvae differ profoundly from those of adults, while in *P. hawaiiensis*, even late embryonic stages already show almost all characteristics typical for adults. This shows that changes in lifestyle and thus in the olfactory landscape a crustacean experiences throughout its ontogeny are reflected anatomically and chemically in the olfactory pathway.

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Clock-related neuropeptides in the pea aphid

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The pea aphid (*Acyrtosiphon pisum*) is a strongly photoperiodic insect whose life cycle is tightly linked to photoperiod. While the involvement of the circadian clock in insect photoperiodism is widely accepted, the neuronal and molecular mechanisms behind remain largely unknown. To elucidate this topic in aphids, a comprehensive characterization of the circadian clock is essential. Our previous studies showed that, similar to *Drosophila*, the clock proteins Period and Cryptochrome are expressed in lateral and dorsal clock neurons of the aphid brain, and that a subgroup of lateral clock neurons contain the neuropeptide Pigment-Dispersing Factor (PDF). However, nothing is known about other neuropeptides associated with the circadian clock. To address this point, we used a combination of transcriptomics, peptidomics, and immunohistochemical techniques. We found that the lateral neurons express the neuropeptides FMRFamide, Orcokinin-A, and Allatotropin, while the dorsal clock neurons contain many different neuropeptides, including Allatostatin A, Diuretic Hormone 31, FMRFamide, and Myoinhibitory peptide. Short neuropeptide F (sNPF) and Ion Transport Peptide (ITP), which represent important peptide components in the *Drosophila* clock were absent from the aphid clock neurons. Thus, the peptide composition in the aphid clock neurons is quite different from that in *Drosophila* and resembles more those of the cockroach circadian clock. Notably, the aphid dorsal clock neurons project towards the insulin-producing cells in the pars intercerebralis, an area previously suggested to play a crucial role in the photoperiodic response of aphids. This further evidence for the importance of the dorsal protocerebrum in photoperiodic responses.

The photoperiod strongly affects gene expression in the large ventrolateral clock neurons (l-LNvs) of *Drosophila melanogaster*

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Using their genetically encoded endogenous clocks animals adjust behavior and physiology to the daily occurring environmental changes, such as day length, temperature, and food availability. In *Drosophila*, the clock in the brain consists of about 150 neurons, of which the large ventral-lateral neurons (l-LNvs) are known to play a key role in the light-input pathway. They promote arousal and activity in response to light, especially in the morning (Shang et al., 2008, PNAS 105; Sheeba et al 2008, J Neurophysiol 99; Fogle et al., 2011, Science 331), and, together with the small ventrallateral neurons (s-LNvs), they delay evening activity under long days (Menegazzi et al., 2017, Curr Biol 27; Schlichting et al., 2020, Curr Biol 29). However, the precise regulation of the l-LNvs neuronal activity is still unknown. In this study, we performed single-cell transcriptome analyses six times per day on the l-LNvs of flies exposed to a long (16h:08h) and short (08h:16h) photoperiod. We found that around 8000 genes are differently expressed under the two photoperiods, some cycling with different phases, others differing in their expression level. We focused on genes encoding neurotransmitter or neuropeptide receptors. Under the long photoperiod, we found a higher expression of the RDL GABA receptor and the GluCl alpha receptor that are responsible for the ON-pathway in the *Drosophila* visual system (Molina-Obando et al., 2019, eLife 8), and the RDL GABA receptor in the l-LNvs additionally in sleep-promotion (Chung et al., 2009, Curr Biol 19). In addition, several excitatory receptors, such as acetylcholine, glutamate and octopamine/ tyramine receptors, were upregulated under long days. Furthermore, the l-LNvs were rich of differentially regulated neuropeptide receptors that may modulate the ionotropic receptors. Overall, the regulation of the l-LNvs appears to be complex. We aim to characterize the function of the different receptors in adapting to day length.

Temperature-induced changes in mitochondrial respiration of *Daphnia magna*

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The habitat of lentic zooplankton poses a challenge due to the seasonal temperature fluctuations and local variations in the water column. In poikilothermic organisms, exposure to varying temperatures affects the properties of mitochondrial function. Metabolic enzymes supplying reduced substrates to the electron transport chain and oxidative phosphorylation system must adjust their activity and flux rates to accommodate altered temperature conditions. Our study found that the respiration of *Daphnia magna* was influenced by the animals' acclimation temperature, with cold-acclimated animals exhibiting higher citrate synthase activity, probably resulting from quantitative adjustments, while warm-acclimated animals displayed a higher thermal sensitivity of the functional properties of mitochondrial enzymes. This suggests that the respiration of *Daphnia* is primarily limited by the provision of reduced substrates rather than restrictions in the respiratory chain complexes. This may help reduce the production of reactive oxygen species and preserve the animals' energy reserves. However, the study also found that elevated temperatures led to an increased defense against oxidative stress, as indicated by the integrated biomarker response.

A draft genome of the neritid snail *Theodoxus fluviatilis*

Laura Iris Regina Fuchs

Universität Greifswald

In light of the ever ongoing climate change and its consequences to species with limited dispersal mechanisms, the ability to respond to novel and changing environmental conditions, either by phenotypic plasticity or genetic adaptation, is pivotal to the longer-term survival. *Theodoxus fluviatilis* (Gastropoda: Neritidae) is an euryhaline snail, which occurs in limnic, as well as brackish water habitats. The dispersal of this species is quite limited, since there is no free-swimming larval stage. In laboratory experiments populations from different habitats were shown to differ in their responses to salinity changes. Moreover, freshwater animals show different survival rates in experiments with different medium salinities than those from brackish water. The reaction norms of freshwater- and brackish water-ecotypes cannot be made to match even by stepwise acclimation of the animals to alien salinities. *Theodoxus fluviatilis* uses compatible osmolytes (amino acids) to balance cell volume during changes in medium salinity. We could recently show, however, that amino acid biochemistry is obviously not a strictly limiting factor for survival during osmotic stress. This indicates that other factors (inorganic ions, ion transporters, skin water permeability) are significantly contributing. Our hypothesis is that the differences in reaction norms between ecotypes are due to genetic differences. In order to study the suspected genomic differences between the ecotypes, we set out to sequence and assemble the genome of *T. fluviatilis*. In cooperation with the Institute of Clinical Molecular Biology in Kiel, we performed whole genome sequencing (PacBio and Illumina). The bioinformatic pipeline resulting in a first draft genome of *T. fluviatilis* will be presented.

Effect of thermal acclimation on lactate dehydrogenase in the water flea *Daphnia magna*

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Daphnia magna is a model organism in fresh water ecosystems and known for its adaptive behavior in changing environmental conditions. The thermal resistance of this aquatic animal is affected by the shift of aerobic metabolism to anaerobic metabolism due to inconsistent supply of oxygen. However, energy supply and capacity to tolerate thermal fluctuations are directly related. In this study, fourteen clones of *D. magna* from Lake Ring in Denmark, reared for years under laboratory conditions, were characterized for their anaerobic capacity at two temperatures, 20°C & 30°C. Anaerobic metabolism may bridge the gap in energy facility via the reduction of pyruvate to L-lactate catalyzed by the lactate dehydrogenase (LDH), which regenerates NAD⁺. The activity of the metabolic enzyme LDH has been investigated for the isoforms by gel electrophoresis and for substrate inhibition using spectro-photometric assays. Interestingly, there has been a significant difference in substrate inhibition among the *Daphnia* clones acclimated at 20°C and at 30°C. Moreover, maximal LDH activity varied in animals from both temperatures. This study gives insight to the anaerobic capacity of *Daphnia* clones, highlighting the physiological response of this species under heat stress. The observed variation of aerobic and anaerobic capacity in *D. magna* at different environmental conditions is helpful in building a forecast model of species persistence under changed climate conditions.

A calcification model construct of a *Rotaliid foraminifera*

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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. To date many of the fundamental problems of biomineralization have not been resolved. Here we present a construct of ideas to the biocalcification process in *Amphistegina 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. The low cellular membrane potential enables the removal of additional protons, originating from production of Mg-ACC, from *Amphistegina* cells. Our studies address a theoretical construct of the understanding of physiological processes involved in marine biocalcification mechanism.

Light entrainment pathways to the circadian clock circuit of the Madeira cockroach *Rhyparobia maderae* controlling sleep-wake cycles

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Endogenous circadian clocks evolved in organisms orchestrating rhythms in physiology and behavior, entrained to the daily light dark cycle. For the night-active Madeira cockroach *Rhyparobia maderae*, transplantation studies located the circadian clock that controls sleep-wake cycles to the accessory medulla (AME) in the brain's optic lobes. The neuronal circuit of the AME in each of the bilaterally symmetric optic lobes of the cockroach brain comprises two negatively coupled oscillator circuits. The morning (M) oscillator is advanced by light pulses at dawn, while the evening (E) oscillator is delayed by light pulses at dusk. All four oscillators of the brain couple with each other to orchestrate together the daily sleep wake cycle. Since the night active cockroach sleeps during the day, the M oscillator appears to be sleep promoting, while the E oscillator is activity promoting.

Neither in the mammalian nor in the insect's circadian clocks it is understood which neuronal mechanisms entrain the neuronal circadian clock network to the daily light dark cycle and how light input into the clock is gated. Since behavioral experiments suggested that UV light is sleep promoting and green light activity promoting, we hypothesized that UV light advances the M and green delays the E clock circuits. To challenge our hypothesis of parallel light entrainment pathways to different clock circuits, behavioral assays were performed in different light regimes. Furthermore, we developed a simplified clock network model based on switched differential equations to describe the oscillatory behavior of four coupled oscillator circuits. Preliminary behavioral experiments showed a tendency of period lengthening in constant green and period shortening in constant UV light, before periods changes slowly reversed. With further behavioral assays combined with model-based analysis, we attempt to refine our hypothesis of interconnections between parallel light entrainment pathways to the M and E clock circuits.

Molecular adaptations of the brain of diving mammals to hypoxia

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Brain energy metabolism is a fine-tuned process, in which astrocytes are active contributors to brain function. Besides modulating blood flow and transmitter recycling, these highly glycolytic cells provide neurons with lactate for oxidative phosphorylation via the electron transport chain. Normal brain function thus relies on the availability of oxygen as electron acceptor and the mammalian brain cannot sustain cellular homeostasis for more than several minutes under hypoxic conditions. In contrast, diving mammals have evolved to cope with recurrent hypoxia during extended foraging dives. Despite well-known physiological adjustments the brain of these mammals repeatedly experiences severe hypoxia and has evolved intrinsic molecular adaptations to ensure its integrity. In vitro electrophysiological measurements conducted by Geiseler et al. (2016) showed that hooded seal hippocampal slices could endure up to 3 hours of hypoxia and still recover after reoxygenation. A recent transcriptome analyses of hooded seal neurons points towards a high metabolic capacity, a reduced intensity of energy-costing synaptic transmission and an elevated antioxidant defense (Geßner et. al, 2022). Considering the tight cooperation between astrocytes and neurons in brain metabolism we now want to investigate astrocyte-specific adaptations in these mammals.

In a first step we will apply single cell RNA sequencing via laser capture microdissection and spatial sequencing to conduct comparative astrocyte transcriptome analyses of marine and terrestrial mammals. Identified differentially regulated candidate genes will then be functionally investigated in neuron and glia cell culture. For this, cloning and knockout of genes will be conducted, complemented by protein assays.

The goal of this project is to extend our knowledge of cellular and genetic mechanisms contributing to hypoxia tolerance in the brain. Since hypoxia is a driver of various human pathological conditions such as Alzheimer's Disease, stroke or tumour development unravelling protecting adaptations of our mammal relatives may contribute to future medical studies.

DUR3-like Urea Transporters of *Theodoxus fluviatilis*

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Theodoxus fluviatilis (Linnaeus, 1758) (Gastropoda: Neritidae) is an oligohaline aquatic gastropod that inhabits most of Europe and adjacent areas of Asia. Two different ecotypes can be distinguished: One in freshwater (FW) and another along the Baltic Sea coast in brackish water habitats (BW). Individuals of either ecotype use free amino acids and urea as organic osmolytes to adjust body fluid osmolality to the external medium, however, the BW ecotype is able to accumulate them in larger quantities. The use of urea as an organic osmolyte in aquatic gastropods such as *T. fluviatilis* has only recently been initially described and raised the question of how urea transport between body fluids and the environment is balanced. Upon examining transcriptome and preliminary genome sequence data of *T. fluviatilis*, we identified putative homologues of DUR3 genes, which code for urea transporters (UTs) in other organisms. Here we provide evidence for the presence of four different subtypes of DUR3-like UTs that belong to two distinct families. Two of the UT subtypes were subject to qRT-PCR analyses to investigate differences in mRNA expression during the acclimation of individuals of both ecotypes to different salinities. Our results indicate that only BW animals regulate DUR3 gene expression in the context of osmoregulation.

Seasonal and spatial transcriptome responses in Elbe estuary ruffe (*Gymnocephalus cernua*)

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Transcriptome analyses provide insights into biological processes at the molecular level as a response to environmental conditions. Many studies on fish have taken place under laboratory conditions focussing on one or a few specific stressors. In recent years, greater attention is being given to examining multiple stressors and assessing unexpected additive effects.

In this study, we investigate gene expression patterns under natural conditions by catching fish along the Elbe estuary between summer 2021 and summer 2022 and taking tissue samples from liver and gill tissues immediately on board.

The liver-transcriptome is meant to give us insides into metabolic processes, whereas the gills perform respiratory, excretory, and immunological functions. In the ongoing process, stress response pathways are identified from differentially expressed genes in RNAseq-datasets from more than 100 individuals in both tissues and compared between locations and seasons within the Elbe estuary.

Preliminary results indicate most differently regulated pathways between the cold seasons (winter/autumn) and warm seasons (summer/spring) and less pathways within the spatial patterns along the steep salinity gradient. Notably, similar pathways are found to be regulated in both gill and liver in the same direction. Special attention will be given to energy consuming pathways and the correlation to physiological measurements to estimate the health status and identify the most prominent abiotic factors in the multi-stress environment of the Elbe estuary on fish health.

A *Drosophila* model to study the development and treatment of Cystic Fibrosis

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Kiel University / Zoology

Cystic Fibrosis (CF) is an inherited disease caused by a mutated CFTR gene, which leads to an ion imbalance. As a result of this imbalance, the main clinical manifestations are obstructions of the respiratory epithelium of the lungs and the absorptive epithelium of the intestines, mainly due to increased mucus production. Even today, the life expectancy of CF patients is significantly shorter than that of healthy people, as there is no cure, only symptomatic treatments. However, research in this area is still in its infancy, which makes it all the more important to advance research in this field. For many epithelial diseases, the fruit fly *Drosophila melanogaster* has become a suitable and well-known model in recent years. Therefore, in this study we focus on (i) mucus production by CFTR knockdown in fly intestinal enterocytes and (ii) the investigation of two specific mucus-producing genes, Mur29B and Muc68D. First, we tested different staining methods to detect the mucus layer in the CFTR knockdown mutants. Fluorescence images with lectin staining using WGA showed a massive increase in mucus formation in the intestinal lumen compared to control flies. In addition, we used TCEP (tris(2-carboxyethyl)phosphine hydrochloride), a disulfide bond reducing drug, and were able to detect a significant reduction in mucus after drug administration. We also used the CRISPR-Cas9 system to generate new fly lines to identify the specific role of the Mur29B and Muc68D genes in mucus formation. We used the pCFD6 plasmid for gene knockdown and the flySAM plasmid for gene overexpression. To further verify the mucus composition and the amount of secreted mucus in the generated fly lines, we raised specific antibodies against Muc68D and Mur29B to evaluate the protein levels by Western blot analysis and/or immunohistochemistry to detect the location of both mucins. First experiments with these lines are currently underway.

Analysis of chemical induced inflammatory effects in the intestine of the red flour beetle, *Tribolium castaneum*

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Preclinical studies of inflammatory bowel diseases (IBD) mainly rely on mouse models. Often, the pathophysiological processes of IBD are induced by adding dextran sulfate sodium (DSS) to the drinking water. Due to DSS-induced impairment of the epithelial barrier function the contact with the intestinal microflora is intensified, resulting in an immune response and inflammatory reaction. The symptoms such as gastrointestinal/rectal bleedings, diarrhoea, rectal prolapse and the associated abdominal pain represent a significant burden for the test mice. Analyzing the pathophysiological processes associated with the loss of epithelial barrier function we have established the red flour beetle, *Tribolium castaneum* as an invertebrate model. We fed larvae with DSS and with the nucleobase uracil, which stimulates the dual oxidase (DUOX) immune pathway in insects, to elicit inflammatory reactions that possibly mimic those observed in mice. In response to DSS and uracil treatment, we found a significant reduction of growth, an increase of mortality and a delay in development. Dissection of the gut revealed signs of inflammation with local thickenings due to epithelial swelling. Furthermore, we found that the permeability of the peritrophic matrix was affected, as 2 MDa dextran particles, which normally retain in the midgut lumen, permeate the peritrophic matrix and enter the surrounding tissue in treated larvae. The expression levels of the immune relevant oxidase TcDUOX as well as of the antimicrobial peptides TcCEC2 and TcATTA1 were increased in response to DSS and uracil treatment, while TcIMD expression was not affected. Interestingly, TcTOLL expression increased in the midgut in response to DSS treatment, but not to uracil treatment. Additionally, we found that the microbiome composition of the midgut changes through DSS and uracil treatment, which can be an indication of dysbiosis. These results may facilitate the use of *T. castaneum* as a high throughput invertebrate model in preclinical studies.

Activity of soluble adenylyl cyclase is required for intracellular pH homeostasis and biomineralization in calcifying cells of sea urchin larva

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Christian Albrechts Universität zu Kiel

Biomineralizing cells concentrate dissolved inorganic carbon (DIC) and remove protons from the site of mineral precipitation. However, the molecular regulatory mechanisms that orchestrate pH homeostasis and biomineralization of calcifying cells are poorly understood. Here we report that the acid-base sensing enzyme soluble adenylyl cyclase (sAC) coordinates intracellular pH (pHi) regulation in the calcifying primary mesenchyme cells (PMCs) of sea urchin larvae. Single cell transcriptomics, in situ hybridization, and immunocytochemistry elucidated the spatio-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 pHi regulation 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 regeneration, establishing a link between PMC pHi regulation and biomineralization. In addition, increased expression of sAC mRNA was detected during skeleton re-mineralization and during exposure to CO₂-induced acidification. Finally, we demonstrated that sea urchin sAC, similar as its mammalian homologs, carries the splicing variants that express only the catalytic domains, and expression analysis revealed that the remineralization process alters the abundance of sAC splicing variants potentially associated with the regulation of sAC activity. These findings suggest that transcriptional regulation of sAC is required to promote mineralization and compensate for acidic stress. The present work highlights a central role of sAC in coordinating acid-base regulation and biomineralization in calcifying cells of a marine animal.

Nitric oxide in the immune and nervous systems of insects

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Arthropods as pathogen vectors are becoming an increasing challenge for human health in times of global warming. Pathogen transmission could be altered by infection through interactions between immune and nervous systems. One effector well suited for information transmission is the gaseous radical nitric oxide (NO), which functions as a signaling molecule in both systems, and can easily cross barriers between tissues.

We study the distribution of NO synthesis in the CNS and immune cells of mosquitoes after bacterial or viral immune challenge. We localise nitric oxide synthase (NOS) by NADPH diaphorase histochemistry and immunofluorescence of the by-product of NO synthesis, citrulline.

We see NOS-positive neurons distributed in most neuropils of the CNS of various mosquitoes, most prominently labelled in the optic lobes and central complex. In the ventral nerve cord, a small number of NOS-positive cell bodies and arborisations in the neuropil could be identified in the abdominal ganglion chain. In the immune system, we found NOS/citrulline positive hemocytes in mosquitoes 24 h after infection with *E. coli*, both accumulated around the heart ostia and distributed over the entire abdomen, in close proximity to the CNS. In control mosquitoes, NOS labelled hemocytes were absent. Furthermore, pericardial cells and a small number of cells in the fat body, probably oenocytes, were strongly labelled.

We compare the distribution of NOS in the CNS and immune cells to the situation in *Drosophila* and in the locust, where we could demonstrate a response of CNS neurons to contact with immune-stimulated hemocytes by increased synthesis of cGMP, the canonical second messenger in NO signaling pathways.

We conclude that NO is likely to play an important role in the mosquito CNS and immune system, and that it is a likely candidate to mediate infection-induced behavioral changes in mosquitos.

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Eurasian freshwater crabs - New insights into relationships and biogeography based on morphology and genetics

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Freshwater crabs of the family Potamidae Ortmann, 1896 [Decapoda: Brachyura] are widely distributed in river systems and lakes of South Asia, Southern Europe and North Africa. They are highly interesting to answer biogeographical questions, because of a special adaption to their life in freshwater conditions. They do not show free swimming larval stages, but hatch as juveniles. For this reason, and because they mainly occur in mountainous areas, their dispersal possibilities are limited and distribution patterns are usually closely linked to geographical conditions. Therefore systematics and biogeography are also highly related.

Since the gonopods and their functional morphology as well as their species-specific interaction with the female gonoducts were used as a morphological feature for the systematics, many new insights into the relationships and the biogeography of potamid freshwater crabs have been gained. However, some fundamental questions, remain unanswered to this day. This applies in particular for the region of the Himalayas where the knowledge and the taxonomical relationships remain poorly known up to now. For this reason, we have conducted new genetic and morphological studies of the gonopods. Next to new collection material from the Himalayas also crab species from the Aegean region the centre of freshwater crab diversity in Europe were investigated. Through these studies we were able to gain new insights into the relationship and biogeography of the Potamidae.

From New Zealand to the world: Analysing the Range Expansion of the New Zealand Mud Snail *Potamopyrgus antipodarum* (Mollusca, Caenogastropoda)

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Invasive species pose a significant threat to aquatic ecosystems, and the New Zealand mud snail (NZMS) stands out among the top 100 worst invasive species in Europe. This study analysed over 400 publications to track the initial occurrences of the NZMS following its introduction to England in 1859, which subsequently led to rapid range expansion across Europe. Similar patterns were observed in Australia (1872) and the US (1987), while the invasion in Japan (2000) and Chile (2011) is relatively recent, hindering long-term estimations. The success of the invading populations can be attributed to various factors, including its parthenogenic and ovoviviparous reproductive nature, phenotypic plasticity, absence of natural predators, and the lack of parasitic interactions, particularly with trematodes. The study traced the invasive trajectory of the NZMS through its first records in Europe, Australia, North America, Japan, and Chile. The extensive dataset obtained from this research serves as a valuable resource for monitoring and understanding the spread of this invasive species. Furthermore, it informs management strategies aimed at mitigating the NZMS's impact on aquatic ecosystems worldwide. Highlighting the NZMS's invasive characteristics and evidence of human-induced introductions, this study emphasizes the importance of predicting its future invasive routes in the coming decades, as the species is anticipated to further extend its range. The research also sheds light on the significant role played by global shipping in facilitating the spread of invasive species, underscoring the urgent need for robust biosecurity measures and enhanced regulation to prevent future ecological disasters.



Infrequent oceanic long-range dispersal and evolution of a top terrestrial arthropod predator in the sub-Antarctic

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Despite their remoteness, isolation and repeated strong glaciation through the Plio-Pleistocene the sub-Antarctic archipelagos harbour a multi-million-year old native terrestrial invertebrate fauna that is pressured by invasive biota and climate change. The biogeographic origins and connectivity between these faunas are poorly understood, yet critical for the conservation of the unique terrestrial Antarctic ecosystems. Here, using phylogenomic tools, we inferred the origins, sequence and timing of trans-oceanic long-distance dispersal of the top arthropod predators in the Eastern sub-Antarctic: spiders of the genus *Myro*. We found that the lineage underwent an adaptive radiation on the Crozet archipelago, from which the Prince Edward Islands, and via the Kerguelen, Heard and Macquarie islands were populated over the last three million years. Together with recent findings on weevils, these results highlight the Crozet Islands' role as an important stepping stone for the colonization of the Eastern sub-Antarctic by macro-invertebrates, and that natural long-distance, trans-oceanic dispersal of terrestrial macro-invertebrates repeatedly occurred along the path of the Antarctic circum-polar current. We discuss the gain and loss of pre-adaptations acting as a filter that enabled only one of four *Myro* species native to the Crozet archipelago to perform such long-distance dispersal under hostile conditions, resulting in an outstanding distribution range over 9000 kilometres. These findings highlight the potential of terrestrial invertebrates to achieve rare but ecologically influential trans-oceanic dispersal events of thousands of kilometres.

Exploring mitochondrial genomes: unravelling phylogenetic relationships and gene order variation within Syllidae (Annelida)

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Mitochondrial genomes play an important role in inferring phylogenetic relationships in animals, offering valuable insights through sequence analysis and gene organization that have been widely used to resolve phylogenies of different taxa at different levels. In Annelida, the mitochondrial gene order was found to be more variable than previously thought, particularly in the Family Syllidae (Errantia), which shows a high frequency of gene order rearrangements. This study uses extensive datasets of mitochondrial genomes from over 20 Syllidae species, along with newly sequenced *Sphaerodorum* sp. (Sphaerodoridae) and *Spinther* sp. (Spintheridae), to conduct new phylogenetic analyses incorporating complete mitochondrial genomes and selected nuclear markers (18S and 28S).

Besides the Pleistoannelida gene order, a total of six different gene orders have been observed within Syllidae. This makes Syllidae and especially Syllinae the group with the highest diversity in mitochondrial gene order within Annelida. Syllidae consistently form a monophyletic group, with *Streptosyllis* sp. (Anoplosyllinae) identified as the sister taxon to the remaining Syllidae. *A. clarae* and *B. lagunae* consistently form a well-supported basal group, suggesting a potential additional independent group with *Dioplosyllis*. Surprisingly, *Autolytinae* was found to be the immediate sister taxa of Syllinae, contradicting previous topologies. However, members of *Exogoninae* and *Perkinsyllis*, traditionally closely associated with Syllinae, are missing from this analysis. Syllinae form a well-supported monophyletic clade divided into the two known subgroups "Clade A" (Ribbon Clade) and "Clade B". Furthermore, the presence of duplicated copies of the tRNA encoding for methionine (trnM) indicates their potential occurrence in all members of "Clade B" as an autapomorphy, providing further insights into the evolutionary dynamics within this subgroup. This study significantly contributes to our understanding of the phylogenetic relationships and remarkable gene order variation in Syllidae, enhancing our knowledge of the evolutionary history within this diverse family of annelids.

Evolution of surface microstructures on leaf insect eggs and their functional significance (Phasmatodea: Phylliidae)

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Leaf insects (Phylliidae) are famous for exhibiting impressive cryptic masquerade. They almost perfectly imitate angiosperm leaves and their eggs often resemble plant seeds. While adult individuals of different Phylliidae species are morphologically very similar, their eggs reveal a significant diversity in shape and surface structures.

Previous studies have documented the specialised attachment mechanism of one species with hierarchical exochorionic fan-like structures (pinnae), which are mantled by a film of an adhesive secretion (glue). Both respond to water contact, with the fibrous pinnae expanding and the glue being capable of reversible liquefaction. Within the whole clade, eggs exhibit surface structures that were suggested to represent specific adaptations to the different environments the eggs are deposited in.

Here, we investigated the diversity of phylliid eggs and the functional morphology of their exochorionic structures. Based on the examination of eggs of all phylliids whose eggs are known, we characterised eleven different morphotypes. We explored the evolutionary history of the different egg types and their adhesive systems by combining molecular phylogenetic methods, scanning electron microscopy and experimental testing of the attachment performance on a broad range of substrates. Furthermore, we tested whether the adhesion is replicable after detachment in multiple cycles. Our results show that the egg morphology is congruent with the phylogenetic relatedness within Phylliidae. The morphological differences are likely caused by adaptations to the specific environmental requirements for the particular clades, as the egg morphology has an influence on the performance regarding the surface roughness. Furthermore, we show that different pinnae types and the presence of glue evolved convergently. While the evolution of the adult morphology of Phylliidae appears to be non-adaptive, the eggs represent a stage with complex and rather diverse functional adaptations including mechanisms for both fixation and dispersal.

Reproductive isolation within a population of *Nasonia vitripennis* (Chalcidoidea, Pteromalidae) caused by single and double infection with Wolbachia

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Wolbachia is a common endosymbiont in many arthropod species. It has multiple implications for the reproduction of its host, including cytoplasmic incompatibility (CI). CI a special form of postzygotic reproductive isolation marked by the absence of offspring from crossings of males infected with the endosymbiont and uninfected females. The genus *Nasonia* (Chalcidoidea Pteromalidae) currently comprises four described species of parasitoids of fly pupae in bird nest or carrion. CI caused Wolbachia represents one of the main reproductive barriers between some of the species.

Here we report on the infection of Wolbachia in a local population of one *Nasonia* species, *N. vitripennis*, in the Park of Hohenheim University, Stuttgart, Germany. In 2012 and 2022, most wasps collected were infected with endosymbionts from the Wolbachia supergroup A. In addition, some wasps were also infected by a second strain from Wolbachia supergroup B. This caused unidirectional reproductive isolation, i.e. the absence of female offspring in crossings of *N. vitripennis* females infected only by the supergroup A Wolbachia strain and males infected by both Wolbachia strains. An infection with the second strain was found in 25% of the wasps tested in 2012, and 50% of wasps tested in 2022, respectively. This may points to a spread of the second strain of Wolbachia in the studied population of *N. vitripennis*.

Together these data provide additional evidence for the hypothesis that an infection with Wolbachia and associated CI can be the first reproductive barrier to arise in a population and therefore might be a starting point for speciation.