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Abstracts

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KN-01

Insect Biotechnology

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Insect Biotechnology, alternatively called yellow biotechnology, has been defined as the use of biotechnology-based approaches to exploit insects or insect-derived molecules, cells, organs or microorganisms for development of products or services for medical, agricultural or industrial applications. This emerging field is developed within the Hessian excellence initiative. The talk provides an overview about the projects funded within the LOEWE center for insect biotechnology and bioresources (http://insekten-biotechnologie.de/en/start.html) representing Europe’s first operational unit in this highly innovative research field with tremendous growth prospects. The center is located at the Justus Liebig University Gießen and operated in cooperation with the University of Applied Sciences in Central Hessen and the Fraunhofer Institute for Molecular Biology and Applied Ecology. The focal points of the center’s work include exploiting insects as a resource for natural substances, producing and formulating these substances, establishing insect models for pre-clinical research and food security, and developing new strategies for combating harmful insects and parasite vectors in an environmentally friendly manner. Basis of insect biotechnology is the in-depth knowledge of the systematics and ecology of insects.

KN-02

C. elegans in an Evolutionary and Ecological Context: Vulva Development and Natural Pathogens

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Biological processes are generally studied in the laboratory under one environmental condition and in one reference genetic background. We try to widen this horizon to answer questions on the relationship between genetic and phenotypic evolution, by placing a paradigmatic model system in developmental biology, \textit{C. elegans} vulval cell fate patterning, in its evolutionary context. We study properties of robustness, evolutionary variation and evolvability of this system.

In order to develop an evolutionary and ecological framework for \textit{C. elegans}, we isolate and study natural populations of \textit{C. elegans} and its relatives. Natural pathogens provide strong and changing selection pressures and are thus relevant to study the defense systems of \textit{C. elegans} and their potentially rapid evolution. Several natural pathogens of \textit{C. elegans} were isolated, including the first viruses that infect \textit{C. elegans} or \textit{C. briggsae}. A genome-wide association study of Orsay RNA virus load after infection of a worldwide set of \textit{C. elegans} isolates indicates one major locus segregating in the species. We found that this major locus corresponds to a widespread deletion inactivating the homolog of vertebrate RIG-I viral sensors, thus allowing viral replication.
Braumeisterin des Lebenden: die Aristotelische physis, unsere „Natur“

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Evolution of Mammalian Diving Capacity Traced by Myoglobin Net Surface Charge

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Several mammalian lineages, such as whales, seals and sea cows, have secondarily entered the aquatic niche and some have evolved the capacity for breath-hold diving in the cold depth of the oceans for more than one hour. This is achieved by elevated body O2 stores and adaptations that maximise the energetic efficiency of aquatic locomotion. While the skeletal modifications associated with the evolution of an aquatic mode of life are relatively well presented in the fossil record, information on the evolution of body O2 stores has been missing. To gain insight into this point, we have recently modelled the maximum active breath-hold submergence time (tmax) of a large subset of living mammals using body mass and the maximal muscle concentration of the key O2 storing protein myoglobin (Mb) as predictors [1]. This allowed predicting more than 80% of the variation in tmax across 42 diving mammals, from water shrew to blue whale. We have further uncovered a molecular signature in the Mb protein, namely its modelled net surface charge, which we show to be closely linked to its maximal expression level in skeletal muscle. Sequencing a large number of Mbs, including from extinct Steller’s sea cow and woolly mammoth, and using ancestral sequence reconstruction, we were able to model the Mb sequence, its net surface charge, and thereby its maximal muscle concentration in the ancestors of today’s diving mammals. Together with fossil body mass estimates we were then able to reconstruct the evolution of tmax across the mammalian phylogeny, gaining insights into the timing and mode of several convergent mamalian land-to-water transitions, including support for amphibious ancestries of elephants, hyraxes and moles.

Arthropod distribution in a tropical rainforest: solving a puzzle in four dimensions?

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The bulk of Eukaryote diversity on Earth is represented by terrestrial arthropods in tropical rainforests. The diversity of arthropod feeding guilds and functional niches is also unparalleled in tropical rainforests. Hence we contend that (1) quantifying the relative influence of local factors affecting the present day distribution of arthropods is a necessary first step towards scrutinizing the global distribution of biodiversity on Earth; and (2) assessing these factors for different taxa and functional groups is crucial for understanding biodiversity and for developing sound conservation strategies for arthropods in tropical rainforests. To date, most studies have focused on a few assemblages (i.e. butterflies, beetles) or lack a design that allows partitioning of the components of arthropod diversity. Here, we use an unusually large dataset, obtained from the San Lorenzo forest in Panama, where the phylogenetic breadth of arthropod taxa was surveyed using 14 structured protocols targeting the soil, litter, understorey, mid-canopy and upper canopy habitats, replicated across seasons in 2003 and 2004. We collected 6,144 arthropod species from 0.48 hectares of forest and extrapolated total species richness of the whole 6,000-hectare forest containing ~25,000 species. To partition components of diversity, we consider (a) spatial turnover among sites, (b) vertical stratification, and (c) temporal variation among repeated sampling intervals. Using vegetation plot and litterfall data, we also contrast distribution patterns between arthropods and plants in this forest. We show that arthropods are more finely distributed than plants and that an unexpected proportion of arthropod diversity is accounted for by vertical gradients. The findings further suggest that models of beta diversity of tropical trees cannot be used as “umbrella” concept for arthropod turnover, even if tree species richness can be used locally to predict arthropod diversity.

From neurogenetic studies in the fly brain to a concept in circadian biology

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Circadian clocks in the brain help animals adapting behavior and physiology to the 24-hour environmental cycles on earth. Neurogenetic studies in the fruit fly Drosophila melanogaster proved not only fruitful in revealing the molecular mechanisms of circadian clocks but also in unraveling their neuronal organization. Two separate but mutually coupled clocks in the brain - Morning (M) and Evening (E) oscillators - appear to control M and E activity bouts of many animals. M and E activity follows dawn and dusk, respectively. In diurnal animals, this leads to a fusion of M and E activity to one activity bout in the middle of the day under short winter days and to a separation of the two by a siesta under long summer days. Overall this adapts the animals’ activity pattern in an optimal manner to the seasons. Using excellent genetic tools, Drosophila researchers have addressed the neural basis of the two oscillators and could trace these to two distinct groups of clock neurons in the brain. Here, I will review the findings that led to our current understanding of the two-oscillator concept from my personal perspective.
KN-07 Talk of Werner-Rathmayer laureate

The smell of death - Necromones in insects and other articulate animals

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The use of Necromones is suggested to serve as a universal survival strategy among articulate animals. Various fatty acids, e.g. oleic and linoleic acid, are said to serve as passive alarm pheromones that are exuded by corpses in order to warn conspecifics of this potential danger. However, this theory of the deterring “smell of death” is controversially discussed and thus reviewed. Different insects and crustaceans were tested for their reaction to corpses and the fatty acids that were declared as necromones.

It was shown that the behaviour strongly varies amongst the different species, an universal pattern of behaviour towards corpses does not seem to exist. Mostly, there also was no correlation between the reaction towards corpses and fatty acids. In contrast to the necromone theory, cannibalism was frequently practiced and the fatty acids partially appeared attractive as well and were associated with food. Due to their omnipresence and their chemical configuration, oleic and linoleic acid in particular are unsuitable to serve as pheromones anyway. Furthermore it can be questioned whether necromones do exist at all. For many insects, corpses are a valuable food source. Corpses thus needn’t be deterring in general. Naturally, articulate animals are able to distinguish dead conspecifics. However, necromones are not the only possible distinctive mark of corpses. Alternative theories should be preferred that suggest the lack of vital signs or the disappearance of typical substances from the cuticles surface to be the real characteristics of dead animals.

KN-08

Collective Behaviour and Collective Intelligence

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Many group-living species exhibit complex and coordinated spatio-temporal patterns from the motion of locust swarms and fish schools to bird flocks, ungulate herds and human crowds. The common property of these apparently unrelated biological phenomena is that of inter-individual interaction, by which individuals can influence the behaviour of others. Individual-based models provide predictions regarding collective processes which we tested in a set of experiments that explore human crowd dynamics and utilise Robofish to manipulate decision-making processes in live shoals of fish. From this work it appears that in some cases individuals in groups are capable of solving cognitive problems that singletons cannot solve. In this context I will discuss the topic of swarm intelligence (or collective cognition) and present a number of case studies from the human domain to show commonalities between animal and human problem solving strategies.
KN-09

Electric fish in the age of genomics: a test of parallel molecular evolution

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Strongly electric fish generate electric organ discharges (EODs) to shock prey or deter predators. Weakly electric fish generate and sense their own electric fields to orient in their surroundings and those of conspecifics to communicate with each other. In all, electric organs (EOs) have evolved from muscle at least six times in fishes. These multiple evolutionary origins allow us to ask whether similar molecular patterns underlie the parallel evolution of an evolutionarily novel trait. First I will demonstrate how a teleost-specific genome duplication provided two copies of a muscle-expressing voltage-gated sodium channel gene. I will show how in two independently derived lineages of electric fish, one of these genes retained expression in muscle whereas the second gene became uniquely expressed in the EO and underwent accelerated sequence evolution presumably generating species-specific EOD waveforms. I will show how the parallel changes in gene expression in these two lineages were likely “predestined” by conditions that occurred millions of years earlier, at the origin of teleosts. In the second part of the talk I will describe a comparative transcriptomic analysis of gene expression in the muscle and EOs of three lineages of electric fish that have allowed us to identify a set of ~30 genes that may be necessary for a muscle to become an EO. Thus, we show strong support for the notion that parallel changes at the organism level may occur because of parallel changes at the molecular level.
OP-BB-IT-01

The neglected sense: Olfactory communication in zebra finches

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Birds in general and songbirds in particular are primarily known for their elaborate songs and/or conspicuous plumage coloration but not for their sense of smell. Probably because of the dominance of the acoustic and visual senses and the absence of any odor-guided behavior, it has been assumed that birds lack a sense of smell. Birds, however, do not only use olfactory cues for foraging and orientation but also use information originating from body odors for social communication. Zebra finches for example have a species-specific odor profile and are able to recognize conspecifics based on olfactory cues alone. Furthermore they are able to distinguish their natal nest from a foreign nest based on olfactory cues. Very recent experiments show that zebra finches recognize kin based on olfactory cues and that kin recognition in zebra finches is not biased by familiarity. This talk will give an overview about the research on olfactory communication in zebra finches in our group and will give an outlook about future directions.

OP-BB-IT-02

Auditory interactions in the night sky: from neurons to communities

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Echolocating bats and moths with bat-detecting ears interact in a predator-prey-relationship that is solely based on acoustic information. In this talk, I will present neuronal and behavioural adaptations in moths for successfully avoiding bat predation as well as the first example of an echolocation counterstrategy to overcome prey hearing. We studied bat-moth-interactions using flight path tracking in the wild, moth hearing using neurophysiological recordings in the field and lab, bat diet based on fecal DNA analysis and perceptual space and movement patterns using bioacoustic modelling.

Moth ears are simple, possessing only 1-4 auditory neurons, and elicit a dual evasive response. Despite their simplicity, these ears are adapted to their species-specific predation pressure posed by their sympatric bat community. In particular, moth hearing is adapted to the call frequencies of the local bat community, and the onset of neuronal activity provides similar temporal safety margins against bat attacks across multiple bat species differing in predation threat. In contrast to the predator-mediated selection pressure on prey, predators generally experience a lower selection pressure to counter prey defences. Consequently, evidence for a coevolved bat counterstrategy to moth hearing had been ambiguous. The barbastelle bat, however, preys almost exclusively on eared moths by emitting low-amplitude calls. These calls are inaudible to moths, suffer from reduced prey detection distance and derived from a high-intensity ancestral state as adaptation to moth hearing. The bat-moth-system thus illuminates functional and ultimate questions of the sensory and behavioural ecology and coevolution of predator-prey-interactions.
OP-BB-01

The adaptive function of male genital titillators in mating and spermatophore transfer in a bushcricket

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In many katydids (Orthoptera: Tettigoniidae) males possess sclerotized internal genitalia, called titillators. Those are inserted rhythmically into the female's genitalia during copulation. The function of the titillators is still poorly understood.

We used the bushcricket *Metrioptera roeselii*, to investigate the function of the titillators. Therefore, mating experiments combined with manipulations of the male’s titillators were performed. We used (1) control males, (2) males with ablated titillator tips (T-tips), (3) males with both titillator arms cut half (T½), (4) males with titillator arms completely removed on one side (T-1), and (5) on both sides (T-2). During copulation, most females stayed motionless. Female struggling behaviour occurred only at a low rate, increasing to 38 percent for females mated with T-1 males. That struggling behaviour did not result in mate separation. Spermatophore attachment was successful in control, T-tips and T½ males, but removal of titillator arms lowered the male’s capacity to transfer the spermatophore correctly. That spermatophore detachment showed no significant relation with female struggling or the number of titillator movements and therefore seems to result only of titillator removal. Moreover, neither copula duration, nor spermatophore transfer success -with the exception of T-2- was affected by titillator manipulation. We conclude that titillators do not function as anchors for mate securing. A sperm removal capacity is also very unlikely, as we found no sperm attached to titillators after rematings of polygamous females. Hence, titillators might be used for stimulation of the females, suppressing female mating resistance and leading to an acceptance of the spermatophore.

OP-BB-02

Mating plug efficacy in a dwarf spider - the role of size and age.

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An defensive male strategy to secure paternity is the production of a mating plug that blocks the female genital opening after mating. The size and persistence of mating plugs in or on the female genital openings are key traits that affect the strength of paternity protection. We investigated the influence of the size of the amorphous plug material (experimentally terminated mating duration as a proxy) and age of the mating plug (time interval between copulations with two successive males) on the efficacy of the plug by analysing the mating success of subsequent males in the dwarf spider *Oedothorax retusus* (Linyphiidae: Erigoninae). Even though a high proportion of subsequent males (82%) tried to mate, only one third were able to copulate, demonstrating that the plugs are effective safeguards against remating. Remating probability was significantly higher after previous short copulations (resulting in a small plug) compared to long copulations (resulting in a large plug). With increasing age, small plugs became more effective, whereas efficacy of large plugs did not depend on their age being equally high over all remating intervals. The observed copulations, however, do not necessarily result in sperm transfer, since sperm masses were found on the plugged female genital area. Our study shows that mating plugs in *O. retusus* effectively impede females from further mating and that mating plug efficacy is shaped by both plug size and plug age.
**OP-BB-03**

**From hormone to pheromone? A female produced antiaphrodisiac reflects hormone titer during parental care**

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In many species with parental care, females are physiologically restrained from producing further young during the period of offspring feeding. During this time, both, females and males benefit from avoiding unnecessary and costly copulations. In previous experiments, we could show that male burying beetles adjust their mating rate according to a female’s reproductive state and mate frequently in the beginning of a reproductive bout, but stop to copulate during the period of offspring feeding. If females were forced to resume egg laying by removing her offspring, males started to copulate again. Furthermore, we found evidence that males do not use the presence or absence of larvae as a cue to adjust copulation rate, but instead use female-produced cues of reproductive state.

During the time of offspring tending females emit the monoterpenoid ester methylgeranate, which is structurally related to juvenile hormone III. In the present study, we show that methylgeranate and JH III not only share the same biosynthetic pathway, but their amounts are also positively correlated. During the time of offspring feeding, when no copulations occurred, both, hormone titer and the amount of emitted methyl geranate were high; when females were forced to resume egg laying, however, hormone titers and methyl geranate quantities decreased. Further experiments strongly suggest that methyl geranate functions as an antiaphrodisiac that honestly reflects female JH III titer, which in turn inhibits egg laying. Thus, our study may provide an excellent example of how the honesty of a chemical signal can be ensured by a shared pathway between the condition that is signaled and the signal itself.

**OP-BB-04**

**Fitness costs of division of labour**

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Division of labour is thought to be the key to the success of social insects. Yet, the fitness consequences of division of labour have not been experimentally explored. Despite potential benefits of division of labour for colony efficiency, worker specialization may constrain flexible task allocation with detrimental fitness consequences under rapid changes in colony demand. We manipulated the division of labour in colonies of the ant *Temnothorax longispinosus* and show that worker specialization is indeed disadvantageous under dynamic conditions posed by the slavemaking ant *Protomognathus americanus*. During slave raids, host colonies composed of generalist workers saved more sexual and worker brood than specialist colonies. Generalists also inflicted more fatalities among slavemakers, without suffering higher worker or queen mortality. Thus, worker specialization is detrimental for colony reproduction and growth under rapidly changing conditions. These fitness costs confirm that division of labour constraints flexibility and functional robustness, central to the long-standing debate on the adaptive significance of division of labour.
**OP-BB-05**

**Interspecific competition shapes complex cognitive abilities: How archerfish outperform other surface-feeding fish in their shared mangrove habitat.**

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In their natural mangrove environment archerfish face a distinct challenge right after shooting down distant aerial prey with a jet of water. How to secure the dislodged food in the presence of other surface-feeding fish that are immediately alarmed by its impact on the water surface? Here we report that archerfish are commonly outnumbered by up to 30 times more interspecific competitors. Furthermore we found that these rivals are much better equipped with mechanosensors to detect surface waves. Nevertheless, we report that archerfish, surprisingly, manage to gain their downed prey in almost every trial. We show that they do this by employing their so-called predictive start, a highly sophisticated behavior that until now has only been described under laboratory and intraspecific conditions. Our high-speed recordings from the wild show that archerfish respond much faster than their numerous rivals and that they are already on a straightened way towards the prey’s future landing point before it actually hit’s the water. Unexpectedly, we additionally found that their competitors are also able to respond to visual cues, but much slower. Our findings thus suggest that the archerfish' head start has to arise out of superior cognitive abilities. These, in turn, may have evolved due to the high competitive and thus evolutionary pressure. Getting a payoff for the energy previously spent on shooting the prey is understandably more crucial for archerfish than for other species that just wait at the water surface. This finding provides an idea of how competitive forces are able to demand the development and implementation of highly specialized, complex cognitive abilities.

**OP-BB-06**

**Searching for a regular pulse in zebra finch song**

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Songbirds are among the few groups of animals that have the capacity for vocal learning, the tool that allowed us to develop language and vocal music. The process through which birds and humans learn their vocalizations as well as the neuronal system which facilitates this process feature a number of striking parallels and have been widely researched. Surprisingly the study of rhythm in birdsong has received little attention until very recently. The analysis of temporal periodicity could supply valuable insights for research into the neuronal mechanisms of vocal learning and performance as well as the biology and evolution of music. This study presents a method for analyzing birdsong for an underlying regular pulse and reports the first results of its application to song of male zebra finches. The analyzed songs were found to match regular pulses in a certain frequency range. Advantages and disadvantages of the method as well as the interpretation and scientific value of the results are discussed.
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Behavioral Biology

OP-BB-07

A New World Perspective on the Swash-Surfing Behavior of Intertidal Gastropods

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Sandy beaches are demanding, physically dominated ecosystems that require special adaptations in their inhabitants. Tidal migrations of gastropods utilizing parts of their bodies as underwater sail to travel with the swash waves provide an example of a specific behavioral adaptation to this environment. Most published studies on swash-surfing gastropods focus on the South-African genus Bullia. However, swash-surfing was first described for snails from the Gulf of Mexico, and several more or less forgotten examples from the Tropical East Pacific have been mentioned in the older literature. Moreover, some monographs on South American intertidal gastropods from the 19th century show the snails in what seem to be swash-surfing postures, but details are unavailable.

Our recent studies of the sensory ecology, tidal migrations, biomechanics, and feeding behavior of swash-surfing snails in beach ecosystems of Costa Rica, El Salvador, Peru, and Texas demonstrate that numerous aspects of the ecology and behavior of Bullia, the South-African textbook cliche of a swash-surfing gastropod, cannot be generalized and transferred to new-world taxa. For example, American swash-surfing snails include suspension feeders and predators that actively hunt, but no scavengers such as Bullia that rely on dead organisms stranded on the beach. As a consequence, olfactory senses seem to play a negligible role in the tidal migrations of American species. In this contribution, we will present results from our current research, focusing on the morphological diversity of the ‘underwater sails’ used by different taxa, and its biomechanic consequences in an ecological context.

OP-BB-08

Ambush predators may select for small group size in rainforest primate prey

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Classic ecological models of social groups suggest that increasing group size typically leads to a decrease in both individual predation risk and net food intake, with optimal group size being a compromise between these benefits and costs. Among rainforest primates, the main antipredator benefits of sociality are thought to result from the dilution effect and collective detection of predators. However, recent research suggests that vegetation density in rainforest habitats limits the benefits of collective detection against ambush predators such as raptors, felids, and snakes. Further, while larger groups are acknowledged to be more conspicuous to predators, it is widely assumed that this cost is unlikely to outweigh dilution benefits. Here we show in a simple model that per-individual rates of both encounters with predators and successful ambush attacks per encounter can increase with group size, under conditions likely to hold for many primate groups (when increases in group size lead to increases in group spread, conspicuousness, and daily travel distance). Consequently, individual risk against ambush predators that employ a sit-and-wait strategy to search for prey, such as many snakes and some raptors, is lowest in small to medium-sized groups. In contrast, individuals in relatively large groups are favored against ambush predators like felids that employ a cruising strategy to search for prey, although even in this case increasing group size above some threshold increases individual risk. These results suggest that maximum group size among primates can be limited by increasing predation risk. Research focused on primate predators is needed to determine the extent to which the model accurately reflects their behavior.
Behavioral Biology

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OP-BB-09
The long reach of a parasite in ant societies
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The consequences of parasite infections are well-studied on the individual level, but the potentially complex outcomes on social groups are less clear. Insect societies could buffer individual-level effects, but parasites could also try to extend their influence to non-infected members by chemical or behavioral manipulation. Parasite-induced alterations of host phenotypes can be either explained by the parasite’s interest to survive and to increase its transmission, by the host trying to limit infection costs, or they could be non-adaptive by-products of infection. The tapeworm *Anomotaenia brevis* causes distinct changes in its intermediate host, the ant *Temnothorax nylanderi*. Infected individuals show a rather inactive behavior, have a lighter coloration and changed chemical profile. However, they are accepted and well-cared for in their colony, and even seem to have an increased longevity. To explain the tolerance of infected nestmates, we experimentally manipulated the status of field colonies by adding and removing infected workers, and determined colony aggression towards infected and healthy non-nestmates. Our results reveal that the current parasitation status clearly influences the colonies’ response to intruders. Parasitized colonies behaved less aggressive towards non-nestmates and this change in colony-level behavior is based on lower aggression in non-infected workers. Our findings are consistent with colony-level changes in aggression with parasite presence and extended to non-infected nestmates, possibly to facilitate acceptance of infected workers in the nest.

OP-BB-10
Localization of artificial flatfish breathing currents by harbor seals (*Phoca vitulina*)
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Harbor seals are opportunistic feeders, whose diet consists of invertebrates, pelagic and benthic fish, such as flatfish. Flatfish are often cryptic while buried in the sand, but emit a strong water flow from their gill openings (breathing current). We hypothesized that harbor seals are able to detect and localize flatfish using their hydrodynamic sensory system (vibrissae). To test this hypothesis we created an experimental platform where an artificial breathing current was emitted through one of eight different nozzles as a hydrodynamic stimulus. The artificial breathing current mimicked breathing currents that had been measured in flatfish, and was emitted at an angle of 45° to the horizontal. The position of the active nozzle was randomly changed. Two seals were trained to search for the active nozzle and station there for 5 seconds as a positive response. Half of the trials for each seal was conducted with the seal being blindfolded with an eye mask. In both cases (mask and no mask trials) both seals performed significantly different from chance. Due to their characteristic search patterns both seals crossed the nozzles with the hydrodynamic stimulus from different directions. Direction did not affect the seals’ performance. Furthermore, both seals responded to the artificial breathing currents by directly moving the snout towards the opening from which the hydrodynamic stimulus was emitted in most cases of the mask trials (69 % and 75 % for the two animals). Thus they were also able to derive directional information from the hydrodynamic stimulus. Swimming speed of the seals and background noise were also considered in this study since these are aggravating factors seals in the wild have to face during their foraging trips.
OP-BB-11

**Behavourial thermoregulation by European bison in a low mountain range forest in Germany**

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**Introduction and Question:**
The European bison (*Bison bonasus*) is the largest herbivore in Europe. It became extinct in the wild in 1927, but survived due to captive breeding based on 12 founder animals. Large free-living populations are established in Eastern Europe and Asia, but no comparable populations are situated in more densely populated regions.
The first reintroduction project in Western Europe was accomplished near Bad Berleburg, Germany. In 2013, eight animals were released into a commercial forest of low mountain range. Prior to reintroduction the animals were kept in an 89 ha enclosure.
Bison are well adapted to harsh climates, but little is known about the animals’ behavioural thermoregulation within the thermoneutral zone (roughly between -20 °C and +20 °C).

**Methods:**
We measured air temperatures using 25 i-button data loggers with temporal resolution of one hour distributed over the enclosure for one year. The animal’s spatial behaviour was recorded by GPS-telemetry collars.

**Results:**
Three different types of weather situations were identified when isochronal temperatures differed within the area by >4.5°C. During these situations the animals did not alter habitat choice in summer but avoided comparatively coldest places of the home range in autumn and spring when average temperatures were low, but still within their thermoneutral zone.
During winter we found no evidence for such behaviour, probably due to feeding management.

**Conclusion:**
These results indicate that European bison show behavioural thermoregulation even within their thermoneutral zone. This issue should be regarded in management practices, e.g. by providing sand bashes, shelter or shade even at moderate ambient temperatures.

OP-BB-12

**A functional role of the sky’s polarization pattern in orientation in the greater mouse-eared bat**

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Animals can call on a multitude of sensory information to orient and navigate. One such cue is the pattern of polarized light in the sky, which for example can be used by birds as a geographical reference to calibrate other cues in the compass mechanism. The greater mouse-eared bat, *Myotis myotis*, has been shown to calibrate a magnetic compass with cues at sunset. Here, we demonstrate that this bat uses polarization cues at sunset to calibrate a magnetic compass, which is subsequently used for orientation during a homing experiment. This renders bats the only mammal known so far to make use of the polarization pattern in the sky. It is intriguing, as currently there is no clear understanding of how this cue is perceived in this taxon.
OP-BB-13

Stability of behavioural syndromes over the lifetime of a holometabolous insect

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Up to now, only few studies investigated the development of behavioural syndromes or personalities over the lifetime of insects. Particularly, the changes or consistencies, respectively, of behavioural traits across ontogeny of holometabolous insects are almost unexplored. We studied several behavioural parameters at five different time points in life of the holometabolous mustard leaf beetle Phaedon cochleariae (Coleoptera: Chrysomelidae), two times in the larval (second and third larval stage) and three times in the adult stage. We could find two behavioural syndromes in every life stage of P. cochleariae, activity and boldness. In comparison to adult beetles, larvae were less active. This finding is probably related to their different morphology and/or to a possible niche shift which may occur after metamorphosis. Additionally, younger larvae were bolder than all subsequent life stages. Similar results of a decrease in boldness over ontogeny have been previously reported in firebugs and crickets. Furthermore, in line with the pace of life syndrome, more active young larvae developed quicker. Over the adult lifetime of the beetles, the behavioural syndromes remained consistent, with females being more active than males. This might be explained by higher food requirements of females, their need to find appropriate oviposition sites and consequently a higher searching/activity effort. In conclusion, our study demonstrates that the behavioural syndromes, activity and boldness, are stable over the lifetime of a holometabolous insect. Moreover, differences in activity and boldness are related to the age and specific challenges an individual faces at this stage.

OP-BB-14

Effects of parental and own early developmental conditions on the phenotype in zebra finches (Taeniopygia guttata)

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The performance of an individual can be critically influenced by its experience early in life as well as trans-generationally by the conditions experienced by its parents. However, it remains unclear whether or not the early experience of parents and offspring interact with each other and adapt offspring when the parental and own early environmental conditions match. Here, zebra finches (Taeniopygia guttata) that had experienced either early low or high nutritional conditions raised their offspring under either matched or mismatched nutritional conditions. Parental and offspring early conditions both separately affected the offspring’s adult phenotype, but early conditions experienced by parents and offspring did not interact as predicted. Offspring that grew up under conditions matching those their parents had experienced did not do better than those that grew up in a mismatched environment. Thus, transgenerational effects remain a lifelong burden to the offspring acting in addition to the offspring’s own early life experience. The lack of evidence for adaptive programming to matching environmental conditions may result from non-predictive environments under natural conditions in such opportunistic breeders.
OP-BB-15

Why primates concern us all - a novel theory of primate social evolution and its implications for behavioural ecology

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Primate societies show remarkable variation with respect to their size, composition and kin-structure, and differ greatly in the level of cooperation within groups. Owing to this variation, primates have traditionally played a prominent role in social evolution research, and the field of primatology has provided many important insights for the greater field of behavioural ecology. In recent years, however, the exchange of ideas between the fields has somewhat abated; one reason being, that the field of primatology is currently still lacking a formal theoretical basis. I provide an overview of my ongoing work, which draws on theoretical developments in the greater field of behavioural ecology to develop a mathematical theory of social evolution, applicable particularly, but not exclusively, to primates. I adopt an inclusive fitness approach to model costs and benefits of social living deemed to be important in primate social evolution (e.g., communal defence of territories), but which have so far been neglected in formal theory. My work has derived several novel predictions, linking variation in (primate) social systems to variation in life-history. Moreover, I show how taking a primatologist’s view of social evolution has provided novel insights for the greater field of behavioural ecology as well. My work facilitates comparisons of primates with other social taxa. In this way, it allows primatologists to draw on theoretical advances in behavioural ecology, and it allows other behavioural ecologists to draw on the wealth of knowledge and data available for the presumably best studied group of social vertebrates.

OP-BB-16

Impact of social environment on juvenile performance in a cichlid fish

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The social environment provided by conspecifics is assumed to modify the selection pressure on the individual and thus may greatly influence individual fitness. Here, we examine the impact of social environment on juvenile performance in *Pelvicachromis taeniatus*, a West African cichlid fish with biparental brood care. After some weeks of parental care, independent juveniles leave their parents and form loose shoals. In our study, juveniles reared in isolation, i.e. deprived of any social contact since egg stage, showed reduced growth, a different social behaviour and worse anti-predator behaviour relative to group-reared juveniles. Furthermore, we found evidence that the composition of the social environment matters as well. When given the choice between two shoals differing in genetic relatedness juveniles preferred to shoal with kin. Kin preferences seemed to depend on environmental conditions and deceased with increasing risk of kin competition. Shoals consisting of only kin showed better growth compared to shoals consisting of individuals of mixed relatedness. Furthermore, related juveniles formed tighter shoals and were more cooperative during predator inspection compared to unrelated juveniles. Our results indicate that the social environment affects individual performance and that individuals may increase fitness by their behavioural decisions.
**OP-BB-17**

**Social influences on the foraging behavior of Neotropical nectar-feeding bats (*Glossophaga soricina*)**

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The foraging behavior of social animals can be facilitated by passive information transfer between knowledgeable and naïve conspecifics. However, the presence of conspecifics can also decrease foraging success. These positive and negative effects can influence males and females of any given species differently. We conducted two experiments to study the influence of conspecifics on foraging behavior of the nectarivorous bat *Glossophaga soricina*.

1) In a demonstrator-observer experiment, naïve *G. soricina* had to find the only rewarding flower in an array of many unrewarding flowers. We quantified the search effort of focal bats in three different test situations: alone (trial-and-error), with naïve conspecific (social facilitation) and with a demonstrator trained to find the rewarding flower (social transmission). When a demonstrator was present, the focal bats’ search effort was significantly reduced, while there was no discernible difference in search effort between the trial-and-error and social facilitation situation. We thus conclude that passive information transfer can enhance the foraging efficiency of both sexes.

2) Alert calls are frequently produced when encountering conspecifics at feeding sites. In a playback experiment, males were repelled by alert calls, whereas females were attracted by them. When benign social calls were broadcasted, females were attracted again whereas males were neither attracted nor repelled. Our results indicate that female *G. soricina* prefer to forage in the vicinity of conspecifics regardless of potential aggressive interactions. Males, on the contrary, rather forage by themselves than in the vicinity of potentially aggressive conspecifics.

**OP-BB-18**

**Transgenerational effects of the social environment on endocrine profiles and behaviour in Japanese quail**

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The social environment is known to influence an individual’s behaviour and physiology. In birds, independent studies have shown that social density affects both circulating hormones in breeding females and hormone deposition to the yolk. Yolk hormones influence the development of the offspring and represent an important pathway through which maternal effects are established. In this project, we combined the above aspects to further explore in which way the social environment influences hormone mediated maternal effects and offspring phenotype across several generations of Japanese quail (*Coturnix c. japonica*). The two treatment groups in the parental generation consisted of one female and one male (pair treatment) or three females and one male (group treatment) housed together. The F1 offspring generation was housed under social conditions that either matched or differed from the maternal treatment. In each generation we performed behavioural (e.g., emergence test, tonic immobility) and physiological (e.g., stress protocol, yolk hormones) tests to correlate endocrine and behavioural profiles between mothers and daughters. Yolk testosterone allocation differed between the treatment groups, as did plasma testosterone levels. Moreover, we have found effects on F1 offspring behaviour and growth. These results suggest that the social environment induces differential maternal effects, potentially preparing the offspring for the future environment.
OP-DB-IT-01

**Myoblast fusion in *Drosophila*: Cellular and molecular insights into muscle development**

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Myoblast fusion is a fundamental process for the formation of multinucleated skeletal muscles during embryonic development, but is also required after birth during muscle growth and regeneration. The larval body wall muscles of *Drosophila* arise by the fusion of founder myoblasts (FMs) and several fusion competent myoblasts (FCMs). The fusion of both myoblast-types is an ordered set of specific cellular events including cell migration, attraction, recognition, adhesion, F-actin foci formation and membrane breakdown as a result of lipid bilayer fusion. The recognition and adhesion of FMs and FCMs is mediated by members of the immunoglobulin superfamily (IgSF) forming a signalling centre with an F-actin rich core at cell-cell contact points. By using genetic interaction studies, we could show that F-actin formation depends on the nucleation promoting factors Scar and WASp that act differently in Arp2/3 complex activation during fusion. Furthermore, we found that the Scar-complex member Kette is required for the communication between Scar- and WASp-dependent actin polymerization. Recent data also suggest that N-cadherin is involved in the recognition of myoblasts. Unlike the IgSF proteins that move radially from the site of fusion to restrict the fusion area, N-cadherin needs to be internalized from the site of fusion to reduce membrane distance.

OP-DB-IT-02

**Protostomy? Deuterostomy? - Does it really matter?**

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Over 100 years ago Karl Grobben divided all bilateralsymmetric animals into two groups the Protostomia and the Deuterostomia. This major split has been based on the differences in the fate of the blastopore, the embryological opening through which the endomesoderm is internalised. Although recent molecular phylogenies recover this basic subdivision of the Bilateria, it has been known since Grobben’s proposition that the fate of the blastopore is highly variable between animal embryos. While all deuterostomes consistently form the mouth separate from the blastopore, protostomes show variability in the fate of the blastopore ranging from protostomy, deuterostomy and blastopore closures. I address this issue from a embryological and molecular perspective and will provide new developmental data from different protostome taxa that suggest that deuterostomy is ancestral for all Bilateria and that protostomy has been evolved multiple times independently.
OP-DB-01

Filopodia based Wnt transport during vertebrate tissue patterning.

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Paracrine Wnt/β-catenin signalling is important during developmental processes, tissue regeneration and stem cell regulation. Wnt proteins are morphogens, which form a concentration gradient across responsive tissue. Little is known about the transport mechanism for these lipid-modified signalling proteins in vertebrates. We analysed the transport mechanism for Wnt/β-catenin signalling in zebrafish and provide first evidence that Wnt8a is transported on short, actin-based filopodia in order to contact responding cells and activate signalling during neural plate formation. Cdc42/N-Wasp regulates the formation of these Wnt positive filopodia. Enhanced formation of filopodia increases the effective signalling range of Wnt by facilitate spreading. Consistently, reduced filopodia lead a restricted distribution of the ligand and a limited signalling range. By simulation we provide evidence that such a short-range transport system for Wnt has long-range signalling function. We show that a filopodia-based transport system for Wnt8a controls antero-posterior patterning of the neural plate during vertebrate gastrulation.

OP-DB-02

Axis Patterning by BMPs: Cnidarian Network Reveals Evolutionary Constraints

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BMP signaling plays a crucial role in the establishment of the dorso-ventral body axis in bilaterally symmetric animals. However, the topologies of the BMP signaling networks vary drastically in different animal groups, raising questions about the evolutionary constraints and evolvability of BMP signaling systems. We show by loss-of-function analysis and mathematical modelling that two signaling centres expressing different BMPs and BMP antagonists maintain the secondary axis of the sea anemone Nematostella. We demonstrate that BMP signaling is required for asymmetric Hox gene expression and mesentery formation. Computational analysis reveals that network parameters related to BMP4 and Chordin are constrained both in Nematostella and Xenopus, while those describing the BMP signaling modulators can vary significantly. Notably, only chordin, but not bmp4 expression needs to be spatially restricted to lead to robust signaling gradient formation. Our data provides an explanation of the evolvability of BMP signaling systems in axis formation throughout Eumetazoa.
OP-DB-03

Distribution of sea anemone cell types challenges germ layer homology

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Cnidarians (e.g. sea anemones, jellyfish) develop from two germ layers, the outer ectoderm and the inner endoderm, while bilaterian animals (e.g. flies, worms or vertebrates) possess in addition the mesoderm, a third germ layer in between endoderm and ectoderm. It is commonly assumed that the cnidarian endoderm (or ‘endomesoderm’) shares a common evolutionary origin with both the bilaterian endoderm and mesoderm. In order to test this hypothesis, we characterised the localisation of digestive and insulinergic tissue, and their embryonic origin in the sea anemone *Nematostella vectensis*. We found that in *N. vectensis*, but also in the moon jellyfish *Aurelia aurita*, the *foxA*-expressing pharynx and gastric filaments harbour all digestive gland cells and the majority of insulinergic cells. These *foxA*-expressing digestive tissues are thus reminiscent of bilaterian endodermal midgut. Furthermore, the larval pharynx of *N. vectensis*, developing into these digestive tissues, shares a combination of transcription factors with the developing vertebrate endodermal pancreas. Strikingly, long-term cell tracking using embryonic transplants of transgenically marked tissue shows that all digestive tissue of *N. vectensis* is of ectodermal origin. The endoderm of *N. vectensis*, in contrast, harbours muscle cells, gonads and nutrient storing cells with transcriptional profiles very reminiscent of bilaterian mesoderm. This allows us to propose a new concept of germ layer evolution where bilaterian endoderm shares no common ancestry with cnidarian endoderm, but with the pharyngeal ectoderm.

OP-DB-04

FGFR signaling in Hydra: a first, differentially expressed FGF

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FGF receptors are ancestral molecules which evolved in the last common ancestor of cnidaria and bilateria and are functionally highly conserved (1,2). In *Hydra* FGFR signalling is essential for boundary formation and bud detachment (2, 3, 4), but the ligands of *Hydra* FGFR are still uncharacterized. FGFs in other animals are essential to attract FGFR-expressing cells to certain regions and thus control cell migration. Moreover, they are able to act in a concentration-depending manner also in differentiation control (8). We now present data for five FGFs recently identified in *Hydra* databases (5 - 7) as potential ligands. One of them, FGFf is expressed differentially and at high levels in all terminal regions and at boundaries that have to be crossed by cells. This expression pattern suggest an involvement in chemoattraction and guidance of cells towards the termini, or alternatively an involvement of FGFf in terminal differentiation and/or cell shape changes occurring in these regions.

(1) Rudolf et al., 2013; Rebscher et al., 2010
(2) Sudhop et al., 2004; (3) Münder et al., 2010; (4) Böttger and Hassel, 2013;
(8) Bae et al., 2012
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OP-DB-05

Hox genes in Brachionus plicatilis (Rotifera): formation of a miniature nervous system

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Hox genes play key roles in axial patterning and body plan regionalization. Their spatial expression along the anteroposterior body axis and temporal activation sequence during development often reflect the structural organization of the genomic Hox cluster. So far, little is known about Hox gene expression in ‘platyzoan’ taxa, comprising platyhelminths and gnathiferans.

Rotifers are microscopic aquatic eutelic gnathiferans with non-spiralian cleavage and direct development. Special morphological features are the syncytial epidermis with an intracellular lamina, the corona with ciliary bands used in locomotion and food particle collection, and trophi, jawlike articulated structures formed by chitinous elements located in their muscular mastax. In spite of their small size these animals also feature a rather complex nervous system.

Focusing our efforts on the monogonont rotifer Brachionus plicatilis, full-length sequences of transcripts of Hox genes belonging to five paralogous groups have been isolated: an anterior class Gene, a Hox3-class gene, and three central class Hox genes. Contradicting previous studies, analyses of Hox signatures, including regions flanking the homeodomain, point towards a close relationship to chaetognaths. Expression of Bp-Hox genes has been studied in embryos, stages close to hatching, and adults. Activation is restricted to the forming nervous system. Analysis of Bp-Hox expression and immunohistochemical staining of neuronal structures enabled us to identify and visualize the shape of parts of the rotifer nervous system not shown before. This include a caudal ganglion and varying subsets of cells in the forming foot that express Hox genes, suggesting sensory functions of the foot.

OP-DB-06

Neural development in the water bear Hypsibius dujardini (Tardigrada)

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Tardigrades are microscopic invertebrates that live in permanently or temporarily wet environments. They are widely accepted as a part of the Panarthropoda (Onychophora + Arthropoda + Tardigrada) and therefore comprise an important outgroup for understanding arthropod relationships. In particular, the position of myriapods is controversial, as they are regarded as either the closest relatives to chelicerates (Myriochelata hypothesis) or to crustaceans and hexapods (Mandibulata hypothesis). In order to gain insight into this relationship, we examined the origin and development of the nervous system in the eutardigrade Hypsibius dujardini Doyère, 1840 using immunohistochemistry on whole-mount and cryosectioned embryos. Our results indicate that the mode of neurogenesis in H. dujardini is more similar to that of crustaceans, hexapods, and onychophorans than to that of chelicerates and myriapods, since clusters of post-mitotic, immigrating cells are not evident. This finding therefore supports the Myriochelata rather than the Mandibulata hypothesis. Furthermore, we found that the tardigrade nervous system develops from anterior to posterior, beginning with the dorsal brain commissure. The development of the circumoral nerve ring, ventral nerve cords, and trunk ganglia soon follow, while no evidence of a subpharyngeal ganglion is found. This anterior-to-posterior pattern of neural development is similar to that of onychophorans and arthropods, suggesting that this arrangement was present in the last common panarthropod ancestor.
OP-DB-07

Analysis of embryonic eye development in the spider Parasteatoda tepidariorum

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The visual system is among the most important organs that facilitates the interaction of an organism with its environment. Spiders, like most arthropods, possess two independent visual systems, generally termed lateral and median eyes, respectively. These systems have a different developmental origin, are determined by different genes, show different morphology and function and, finally, have strikingly different evolutionary trajectories.

We aim to reveal the embryonic origin of the median and lateral eyes respectively by analyzing the temporal and spatial expression of conserved eye development genes in relation to well characterized anterior head markers in the spider Parasteatoda tepidariorum. We show comprehensive expression data from the P. tepidariorum orthologs of the retinal determination network genes. Furthermore, we show differences in the developmental timing of the median and lateral eyes by analyzing the expression of Pt-rhodopsin1 as well as the head morphology of the developing embryo.

OP-DB-08

Evolution of BarH transcription factors in embryonic development

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BarH transcription factors are members of the Bar class of homeobox genes and part of the highly conserved group of Q50-Homeoproteins. Homologues of the BarH genes were first identified in Drosophila melanogaster (Dm) and also found in a number of vertebrates. Homeodomain transcription factors can bind to a variety of downstream targets, whereas specificity is mediated by post-translational modifications and co-factor interactions depending on the cellular environment. This fine-tuning of transcription factor specificity serves as a versatile toolkit for functional evolution. Fittingly, aside from their conserved function in cell fate determination during retinal neurogenesis in Dm and vertebrates, BarH genes show various unique functions in distinct species. These include tarsus segmentation in distal leg of Dm, cell survival in either ear or mesoderm development in mouse or Xenopus respectively, as well as cell migration in the mouse cerebellum. Spiders are basal arthropods and might represent an evolutionary interstage comparing vertebrates and derived arthropod species like Dm. We found two homologues of BarH genes in the spider Parasteatoda tepidariorum (Pt), which are early expressed in the head region and later in the gnathedite and as small spots in the legs of the embryo. Knockdown of one of the Pt-BarH homologues resulted in different head phenotypes, either missing the complete head region, only the pedipalps or the first walking leg, but no alterations in gnathedite development or leg segmentation. Further analyses of these phenotypes and comparisons with findings from Dm and vertebrates will help to understand the evolution of homeodomain specificity.
OP-DB-09

A single FGF-receptor controls development of the mesoderm and the tracheal system in the beetle embryo.

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Fibroblast-growth-factor (FGF)-dependent cell-cell signalling is required for a variety of processes during embryonic and adult life in vertebrates and invertebrates. In \textit{Drosophila} the two FGF-receptors Heartless and Breathless function independently from each other. During mesoderm differentiation, the FGF8-like molecules Pyramus and Thisbe represent the exclusive ligands for Heartless and the FGF Branchless interacts selectively with the receptor Breathless to control trachea formation.

We ask here whether the single FGF-receptor Tc-Fgfr present in the genome of the red flour beetle \textit{Tribolium} is able to integrate the function of Tc-FGF8 and of Tc-Branchless during embryonic development.

We show that the formation of the mesoderm and the heart is severely disturbed in both Tc-fgf8- and in Tc-fgfr-RNAi embryos. In addition, Tc-fgfr-RNAi embryos show defects in trachea formation that have also been observed in Tc-branchless RNAi embryos.

Obviously, the single FGF-receptor in \textit{Tribolium} is indeed able to transduce the signals from these two different FGF-ligands. On the basis of the genomic structure we hypothesize that in \textit{Tribolium} different FGF-receptor isoforms with distinct binding properties for the respective FGFs can be generated through differential splicing.

OP-DB-10

Investigating the Role of DNA Methyltransferase 1 (\textit{Dnmt1}) in \textit{Tribolium castaneum} via Knock-down and Expression Analysis

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Epigenetic regulation enables rapid phenotypic adaptation of organisms to environmental influences. DNA methylation, an important epigenetic mechanism, is generated by an evolutionarily conserved family of enzymes, so called DNA methyltransferases (DNMTs). The ancestral DNMT toolkit consists of three enzymes DNMT1, 2 and 3. However, the evolution of DNA methylation systems has generated great variation in the sets of Dnmt genes between species. For example, \textit{Drosophila} has lost DNMT1 and 3 and therefore lacks a functional DNA methylation system, while honeybees possess all three DNMTs, and DNA methylation is crucial for caste determination.

We investigated the status of a functional DNA methylation system in the Red Flour Beetle \textit{Tribolium castaneum}, which has overall methylation levels of 0.1 % and possesses \textit{Dnmt1} but lacks \textit{Dnmt3}. Bioinformatic analysis of the genome of \textit{Tribolium castaneum} did not reveal any CpG depletion, in contrast to the analysis of the honey bee, \textit{Apis mellifera}. In the present study, we show that DNMT1 is essential for early embryonic development. Parental RNAi knock-down of \textit{dnmt1} in the mothers caused a developmental arrest in the offspring embryos. This was not observed for male beetles, as the knock-down showed no effect on male fertility. Furthermore, \textit{Dnmt1} is expressed across all life history stages as well as in the reproductive organs of the beetle.

This, to our knowledge, first evidence of a functional role of \textit{Dnmt1} during embryogenesis in \textit{T. castaneum}, calls for further studies investigating additional functions of this enzyme and its role in DNA methylation in the beetle.
OP-DB-11

Adult Prothorax Patterning during Insect Typical Metamorphosis

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Insects are the most species rich taxon on earth. Much of the holometabolous insect biodiversity is generated by changing body form during metamorphosis. During this process, the body shape experiences a dramatical change. Most insects re-use most of the larval epidermis to build the adult epidermis, while some structures are made by imaginal cells. *Drosophila melanogaster* is an extreme case, which only uses imaginal cells. In contrast, *Tribolium castaneum* shows more typical metamorphosis, in that it re-uses larval epidermis for the adult structure. So, it can become a model system to study the shaping of the adult body form. The genome-wide RNAi screen “iBeetle: Functional Genomics of Insect Development and Metamorphosis” is revealing genes required for pattern formation during metamorphosis.

We have identified several genes, which are required for forming the shape of the pronotum. Here, we collected 9 phenotypes affecting the pronotum from iBeetle database, 3 of which were confirmed by injecting non-overlapping dsRNA fragments into another beetle strain. The knocking down of *Apaf-1 related killer* gene in *T. castaneum* (*Tc-Ark*) results in the formation of a median line without bristles on the pronotum. The phenotype of gene *Tc-001035* shows less sensory bristles. Furthermore, the lack of gene *Tc-000401* induces anomalous indentations on the adult pronotum. In order to study these phenotypes in more details, we developed the proper *in situ* hybridization (ISH) approach for our research. We plan to combine the study of cellular process and gene function to elaborate the mechanism of these phenotypes.

OP-DB-12

Identification of the molecular changes underlying head shape variation in three closely related *Drosophila* species

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The size and shape of an organism and its organs has to be tightly controlled during embryonic and postembryonic development to ensure proper functionality. However, these adult features are certainly target for evolutionary changes leading to the breath-taking diversity of body forms observable in nature. This contradiction suggests that developmental gene regulatory networks (GRNs) are constraint to a certain level, but nodes within this network are prone to change to give rise to morphological divergence. One aim of our research is identifying flexible nodes within the GRN underling adult head formation in *Drosophila*.

We previously showed that compound eye size and head shape varies considerably within and between the three sibling species *D. melanogaster*, *D. simulans* and *D. mauritiana*. We use the development of this complex adult organ as a system to study variation on a GRN that overall should be very similar but obviously has evolved to produce significant size and shape differences. To this aim we apply RNAseq to unravel the core transitions in the GRN during eye-antennal imaginal disc development. Subsequently, we identify those genes differentially expressed between species with morphological differences to find candidate nodes of the network prone to evolve. This search for the varying nodes is aided by quantitative trait loci (QTL) mapping what allows a more rapid identification of candidate genes. The results obtained will be discussed in the context of GRN constraints and flexibility.
OP-E-IT-01

Mismatches in seasonal timing between predators and prey: causes and consequences

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Phenology - the timing of life-history events, as flowering, migration, breeding or hibernation - generally has consequences for reproduction or survival, as there is often only a short time window of suitable environmental conditions. An important ecological factor determining this time window is food availability. This means the optimal timing of higher trophic levels, i.e. the predator, often depends on the phenology of lower trophic levels, i.e. the prey. Phenology is strongly determined by ambient temperature and climate change has correspondingly generally advanced phenology. However, phenologies of predators and prey has often advanced at different rates leading to mismatches in seasonal timing. Such a mismatch will lead to selection on phenology of the predator and possibly negative consequences for individual fitness and ultimately demography. Using a combination of empirical work from a long-term study on great tits and theoretical modeling I here show why climate change will invariably lead to selection on phenology, under which circumstances successful adaptation will be possible and that ecological processes, as density dependence, can affect demographic consequences of selection and adaptation.

OP-E-IT-02

Below the surface: How soil biota affect plant-animal interactions and ecological communities

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The relationship between plants and their microbial root symbionts belongs to the earliest interactions in the evolution of terrestrial plants. There is growing evidence that these symbiotic biota (e.g. mycorrhiza and rhizobia) are not only beneficial in terms of plant growth and nutrition but may also importantly affect plant-animal interactions. Whilst it was initially assumed that these effects are mainly mediated by an increased availability of limiting nutrients it is becoming increasingly evident that plant defence is affected by root symbionts via different paths and mechanisms. In this talk I will show that root symbionts will not only influence feeding by insect herbivores via changed nutrients and direct defence mechanisms but also via mediation of indirect defence like volatile production, extrafloral nectar production and predator attraction. I will further present results demonstrating underlying mechanisms ranging from mediations in intra-plant resource allocation to differences in gene expression patterns in plants with and without symbionts and their relation to feeding by herbivorous insects. In conclusion, root symbionts can be seen as an integral part of the plant’s defence system which can be assumed to be a driver of the evolution of plant-animal interactions.
OP-E-01

Effects of anthropogenic habitat disturbance on lemur health: friend or foe?

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Parasite infections play an important role in ecological processes, but the potential drivers of these infections are still unclear. However, habitat disturbance has shown to influence parasite prevalence in several animal species. This study aims to explore anthropogenic disturbance effects on gastrointestinal parasite infections in wild prosimian primates. Methods include the non-invasive collection of faecal samples and individual body condition scores of eight congeneric lemur species (genus *Eulemur*). Faecal samples were screened for parasitic eggs and larvae. Two genuine parasitic pinworms (*Callistoura* and *Lemuricola* spp.) and two protozoa species (*Giardia* and *Cryptosporidium*) were found. Parasite prevalence was higher in lemurs ranging in relatively disturbed areas compared to populations that live in less disturbed habitats ($F_{(1,244)} = 6.98, P= 0.009$). In these areas, the presence of introduced fruit trees appeared to result in considerably higher body condition scores and immune status compared to lemurs ranging in more pristine habitats, where food was relatively scarce ($P< 0.05$). We discuss the unexpected relation between human induced habitat changes and parasite prevalence. Parasites have the potential to influence population viability and to spread infectious diseases amongst wildlife and human populations. Hence, further studies on the interactions between the various drivers of parasite infections are needed for a better understanding of the dynamics and social processes within lemur populations and emphasize the necessity of incorporating these insights into conservation efforts.

OP-E-02

Sustaining trophic functions in tropical ant assemblages.

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Ecosystem function resilience in regards to species richness is a well-studied aspect in primary producers’ however little is known on higher trophic levels such as in invertebrates. Interspecific competition is high in ants, with few species dominating food resources and displacing other species. Therefore, niche differentiation is an important mechanism to maintain the high local species richness, which is typical for tropical communities. Species composition and the ecosystem functions performed by ant communities can drastically change with habitat disturbance. However, detailed information on the functional niches is only available for a few ant species, hampering our understanding of the importance of niche differentiation in ant communities in general. We conducted a comparative study in varied sites in the neo- and paleotropics. We used eight bait types that reflected different natural resources. On a grid system, every bait was offered night and day, in order to estimate niche position and breadth for each ant species. Our results demonstrate that ant communities consist of a range of dietary and temporal specialists and generalists. Functional redundancy increased with ant species richness but not in concordance with the effective functional redundancy, indicating that the alteration of community structure does not invariably lead to a degradation of trophic functions. Some ant species play key roles in structuring the food webs of tropical rainforests and we can show that this is due to their biomass, numerical abundance, type of food specialization and temporal breadth. Finally we demonstrate the importance of temporal and spatial scaling in ecosystem resilience and distinguish parallels between continents.
**Landscape Related Resource Diversity Improves Bee Fitness**

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Global declines in wild and native bees have raised concerns about reliable pollination services to crop plants. The bees in turn depend on the availability and diversity of their key resources (pollen, nectar, resin) provided by various plants. Yet how plant composition and resource diversity on the landscape level affect the foraging behaviour and ultimately colony fitness of social bees remains largely unknown.

We studied long term resource effects on the colony development of the perennial Australian stingless bee species *Tetragonula carbonaria* (Apidae: Meliponini) in an experimental setup. We selected twelve study sites in three landscape types (plantations, forests and gardens) and measured plant diversity for each study site. Resource foraging and intake of colonies was observed over six consecutive seasons, while changes in nutritional quality of pollen and nectar storages as well as overall colony growth and reproduction were monitored over three years.

Although garden sites showed the highest, forests intermediate and plantations the lowest plant and resource diversity, we found foraging patterns to be largely similar across landscapes. Pollen diversity and overall resource intake was highest in gardens but low in forests and plantations. Likewise, nutritional quality of honey was highest in gardens, whereas pollen quality was equally high in gardens and plantations. Colony growth rates and reproduction were consequently highest in gardens, but highly variable in forests and plantations.

Our findings confirm the importance of resource availability, diversity and composition in determining colony growth and hence fitness of social bees. However, highest resource diversity is not necessarily associated with large natural habitats and gardens may have a surprisingly high conservation value for bees.

**Re-establishing the burbot (*Lota lota*) in NRW - combining science and applied water management**

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The burbot (*Lota lota*) is the only cod-like (gadiform) fish living in freshwater. It requires cool and oxygen rich water. Before industrialization it was a very common predator in NRW (North Rhine Westfalia), but due to the deteriorating water quality it became almost extinct. During the last couple of years, however, large efforts have been made to restore the natural state of rivers in NRW and to enhance water quality. As the burbot is an important factor in the river ecosystem (and also an attractive target for fishing) the Landesfischereiverband Westfalen & Lippe has developed a breeding and re-introduction program. Burbots have been bred and raised quite successfully. Being aware of the potential negative effects of inbreeding and overall low genotypic diversity, however, the Landesfischereiverband asked for a genetic analyses and evaluation of their breeding population as well as other populations that might potentially be included into the program. Our microsatellite analyses showed that genotypic diversity and heterozygosity was quite high in the breeding population while other populations showed low levels of genetic diversity and evidence of inbreeding. Based on our results we were able give practical recommendations about including and excluding populations into/from the breeding program. Our project shows that the combination of science and water management can be very productive for both sides.
Linking habitat suitability to demography: a case study on great crested newts (Triturus cristatus)

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Understanding the relationship between habitat suitability and population parameters is central to the monitoring, management and recovery of endangered species. Habitat suitability index (HSI) models are often used to predict species occurrence and are frequently deployed in wildlife management and conservation practice. The HSI is a numerical index, ranging from 0 (unsuitable habitat) to 1.0 (optimal habitat). In the application of HSI models for management purposes, habitat suitability is often used as a general indicator for demographic parameters. However, whether and how strongly HSI is actually related to crucial demographic parameters such as apparent survival and reproduction has rarely been evaluated. Here, we conducted a case study to explore the relationship between the HSI and occurrence and demographic parameters in the great crested newt (Triturus cristatus). This species has suffered severe declines and is strictly protected under the European Habitats Directive, therefore constituting a representative and suitable candidate for the general development of informative and easily applicable tools for the monitoring and management of threatened species. Based on a capture-mark-recapture survey of 22 lentic water bodies over three years, we evaluated the relationship between the HSI and occupancy, reproduction and survival probabilities of 1838 great crested newts. Our results show a tight link between the HSI and occurrence as well as reproduction, thereby confirming that a simple measure such as the HSI can provide an effective tool for rapid population management and prioritisation of valuable habitats particularly worthy of protection.

The burst of wild boar populations in Europe: how local adaptation mediates the effects of climate change in a widespread ungulate

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Although climate change is known to affect ecosystems globally, our understanding of its impact on large mammals is still sparse. Further, there is limited knowledge of the effect of climate change on local populations of widespread species. We investigated large-scale and long-term effects of climate change on local population dynamics using the wild boar (Sus scrofa) as a model species. We show that, across Europe, wild boar population increases over the last 150 years are strongly associated with an increase in average winter temperatures. Additionally, the negative effects of cold winters on population growth can be completely outweighed by beech masting events, which provide important food resources and occur with increasing frequency due to climate change. For the first time, we demonstrate that wild boars are locally adapted to prevailing conditions, as the minimum winter temperature required for a population to grow was lower in colder than in warmer regions. We conclude that physiological trade-offs between seasonal requirements for thermoregulation and energy turnover shape local adaptations, such as a significantly increased body mass in colder regions. Thus, seasonality and local adaptations need to be considered in attempts to predict a species’ response to climate change.
Migration routes and wintering areas of the Common Swift *Apus apus* detected by geolocators

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The Common Swift *Apus apus* is an insectivorous bird species and a long-distance migrant. It spends virtually its entire life on the wing except for a short breeding season in Europe. From a few ring recoveries and field observations, it is known that swifts overwinter in tropical and south-eastern Africa. But to understand life-history decisions, it still has to be clarified which migration routes swifts use and whether there are certain destination areas. The deployment of retrievable geolocation data loggers (geolocators), which record time and light-level data for calculation of time-related geographical positions, provides the reconstruction of migratory routes and determination of wintering areas even in small birds like swifts. We fitted ten adult swifts of a breeding colony located in West Germany with geolocators in 2012 and 2013, respectively. Analysis of data from recaptured individuals revealed that swifts headed for the Congo Basin as first destination area during autumn migration which was already described as wintering ground in a geolocation study of swifts breeding in Sweden. In contrast to the Swedish birds, the swifts of our study population moved on to south-eastern Africa, either to the region of the Lake Victoria (Uganda, Tanzania, Kenya) or to Mozambique, and stayed there until the turn of the year. Single individuals flew even further to the Orange River Basin in South Africa. All individuals gathered again in the Congo Basin before the onset of spring migration along the West African coast. Although our swift tracks showed some similarities to those from pilot studies in England and Belgium, there seems to be no static migration pattern in Common Swifts - probably because swifts use a fly-and-forage migration strategy.

Cannibalism and intra-guild predation in pit-building antlions

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Competition in trap-building predators such as antlion larvae is a complex biotic interaction, potentially involving cannibalism and intra-guild predation (IGP). This complexity is expected to strongly influence population and community dynamics. *Myrmeleon hyalinus* and *Cueta lineosa* are two common pit building antlions in Israel’s Negev desert. The two species are often found in close proximity; however, while *M. hyalinus* prefers inhabiting the more productive sandy soils, *C. lineosa* solely inhabits the relatively poor loess soils. To better understand the mechanisms driving the spatial distribution of these antlion species, we preformed two complimentary experiments aiming to explore the consequences of *M. hyalinus* presence on the survival of *C. lineosa* at varying densities and under different abiotic conditions. We found that the presence of *M. hyalinus* significantly reduced the survival rate of *C. lineosa*; however, this pattern was stronger at high density. In most IGP events (93.4%), *C. lineosa* was the victim. Increased *M. hyalinus* size advantage significantly increased the frequency of IGP; however, this pattern was more moderate in sandy soil combined with low temperature. Similarly, the positive effect of *M. hyalinus* size advantage on the frequency of IGP was more prominent in the shallow loess soil, which constrains the ability of the larvae to evade predation. To conclude, we demonstrate that in contrast to traditional thinking, IGP can be governed by the interactive rather than additive effects of biotic and abiotic factors. The observed asymmetrical IGP on *C. lineosa* may explain why this species is restricted to the relatively poor loess habitats, while *M. hyalinus* is more abundant at the more productive sandy habitats.
More than cuticular hydrocarbons: the high chemical diversity in the courtship pheromones of parasitoid wasps

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Males of the parasitoid wasp genera Asobara and Leptopilina, both larval parasitoids of Drosophila, display high frequency wing fanning to court females. This courtship behavior is characteristic for many parasitoid wasps and is usually elicited by a close range female sex pheromone. In many species cuticular hydrocarbons (CHCs) have been identified as components of the courtship pheromone, but other compounds may also be important.

Here we present data on the courtship pheromone of A. tabida and three species of Leptopilina. Our results demonstrate that the courtship pheromone of A. tabida is characterized by remarkable chemical diversity. A multi-component blend of methyl 6-methylsalicylate (M6M), fatty alcohol acetates (FAAs) and cuticular hydrocarbons (CHCs) elicited male courtship behavior. And only a blend of M6M and FAAs or combinations of one or both of these with female-derived CHCs was sufficient to elicit a full behavioral response in males.

All three investigated Leptopilina species produce iridoids and CHCs, but the compounds are used to different degrees in the courtship pheromone. In L. heterotoma, only iridoids elicited wing fanning in males, while in L. victoriae CHCs were sufficient for the full courtship display. In L. boulardi only iridoids and CHCs together elicited the full courtship behavior compound classes.

Although the closely related parasitoid wasps we considered in this study all possess a similar potential reservoir of compound classes from which to compose their pheromone blends, our results indicate that they use different combinations of compound classes during courtship. Our findings also indicate that CHCs are not always a required component of the courtship pheromone for these wasps.

Understanding the phylogeography of Synchaeta pectinata: Extending geographic data with temporal sampling

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The rotifer Synchaeta pectinata is a common species of the zooplankton of standing waters throughout the world. Their heterogonic reproduction system enables high dispersal via sexually produced resting eggs in combination with fast reproduction via the prevalent reproductive strategy of parthenogenesis. To examine the population structure of S. pectinata in Europe and to search for possible cryptic species, we conducted population genetic analyses using the mitochondrial marker COI. Phylogeographic investigations of multiple populations in northern Germany and northern Italy showed high overall genetic variability, albeit with distinct differences among the populations. On a regional geographical scale, genetic similarity among neighbouring, but isolated populations (e.g., ponds and lakes) in Germany was generally low. Paradoxically, higher genetic similarity occurred on the broader European scale. Temporal sampling in northern Germany, however, hints that this geographic picture might be biased because it is based on a single snapshot. Instead, repeated sampling of the same standing waters reveals high variation and dynamics both within and between populations. Whereas some populations show a relatively constant haplotype structure, others are much more dynamic, either through apparently random changes or possibly some form of ecologically-driven haplotype selection. We conclude, therefore, that longer term investigations are essential to more fully understand the relationship of demographic history, selection and population dynamic of rotifer species.
OP-E-11

Tracking livestock-associated bacteria in the gut-microbiome of sympatric black-backed jackals (Canis mesomelas) in central Namibia

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Human population growth and the related agricultural encroachment lead to an increase in the contact probability of domestic animals and wildlife species facilitating the transmission of livestock-associated bacteria into sympatric wildlife species. Some bacterial taxa might be naturally present in livestock and wildlife species sharing a habitat, but it is unknown whether livestock-associated gut-bacteria are transmitted to wildlife species and integrate in their gut-microbial community. Here, we applied a 16S rDNA next-generation sequencing approach to investigate the gut-microbiome of black-backed jackals living either on wildlife or livestock farms in central Namibia and of cattle from those livestock farms. To examine whether bacterial transmission from cattle to black-backed jackals occurs, we analyzed bacterial sequences of shared bacterial taxa on a species-like level, so called oligotypes. We then screened black-backed jackals from both farm types for the occurrence of oligotypes that were present in cattle. Our results show that cattle-derived bacteria are present in black-backed jackals sampled on livestock farms but not in individuals sampled on wildlife farms. However, the proportions of such oligotypes in black-backed jackals were very small and oligotypes were detected only in some of the shared bacterial taxa. These findings suggest that transmission occurs either at a very low rate or that cattle-derived bacteria are suppressed by the species-specific gut-bacteria of the black-backed jackal. Nevertheless, the presence of livestock-derived bacteria in wildlife gut-microbiomes might not pose a threat to wildlife health as long as these bacteria are not pathogenic or negatively impact nutrient uptake.

OP-E-12

The effect of parental age on development, body mass and resistance to stress

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Parental age often affects offspring performance, and is an example of trans-generational plasticity. In the red flour beetle, we used parents of one to four months old to investigate the effect of aging on the offspring performance. First, we documented the development time, body mass at pupation and body size (wing length). We found that offspring of the oldest parents reached a higher body mass and took longer to develop. Second, we investigated how long the beetles endure starvation, and found that offspring of the oldest parents had the shortest survival. This is surprising because they were the heaviest group and there was generally a positive correlation between body mass and survival. We calculated the rate of mass loss, and demonstrate that offspring of the oldest parents “burn” mass faster. Third, we measured cold tolerance of the beetles (cold-shock recovery time). We expected a slower recovery of the offspring of oldest parents, but detected no differences based on parental age. Fourth, we checked the cold tolerance of the parent generation (beetles of different age), as little is known on the aging consequences for cold tolerance. We detected a humped-shape pattern of cold tolerance, with young beetles (a few days after eclosion) and old ones (four months old) exhibiting the lowest tolerance. Body mass contributed to cold tolerance, but only in these two sensitive stages of early and late age. In short, our study demonstrates some costs aging incurs on offspring and is an example for non-genetic carryover effects from parents to offspring.
OP-EB-IT-01

Genomic basis of a 30 year-long selection experiment for longevity in *Drosophila*

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Evolutionary theory posits that aging evolves by mutation accumulation and/or antagonistic pleiotropy. However, to date, the precise identity of the genes and molecular polymorphisms that underlie the evolution of aging and longevity remains poorly understood. Here, we have analyzed the genomic basis of the evolution of longevity in *Drosophila melanogaster* by applying whole-genome next-generation resequencing to a set of longevity selection and control lines initiated by Leo Luckinbill and Bob Arking about 30 years ago. Using population genetic and statistical tools, we have identified candidate genes whose patterns of differentiation have most likely been shaped by selection. Interestingly, among the candidates, we found strong evidence for a functional enrichment of immunity genes, particularly those involved in the defense response to fungi. To test whether this pattern is functionally relevant and real, we measured survival of selected and control flies upon pathogenic challenge with the fungus *B. bassiana*, the gram-positive bacterium *E. faecalis*, and the gram-negative bacterium *E. carotovora*. We found that long-lived lines are indeed significantly more resistant to all three pathogenic infections than controls. As predicted, the age-dependent decline in survival upon infection with *B. bassiana* and *E. faecalis* was much steeper in control flies than in long-lived selected flies. Moreover, qRT-PCR results further indicated that selection and control lines differ in their age-dependent expression for several immunity genes, including antimicrobial peptides, again confirming functional differentiation between selection and control lines for immunity. Our results thus establish a compelling causal link between the evolution of longevity and improved immune function at old age.

OP-EB-IT-02

Evolution of interaction in a highly social organized environment

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Social organization belongs to a major transition in evolution. Social organized insect, often living in colonies of hundreds to thousands of individuals, are especially prone to various forms of interaction. Here, I distinguish between interaction with environmental factors, which can be abiotic and biotic ones and the interaction between molecules. I briefly illustrate prominent examples of interaction with abiotic factors such as climate and pesticides and the interaction with biotic factors, which includes parasites, microbes and between individuals. The bottom line is, however, the molecular interaction among e.g. proteins affecting regulatory pathway fluctuations. As examples of ongoing and future research projects, I present recent insights into the evolution of the sex determination and the mevalonate pathway.
**OP-EB-01**

**Integrative taxonomy favors divergent evolution of the largest living Odonate species; the Neotropical damselfly *Megaloprepus caerulatus* (Odonata: Zygoptera)**

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Several outstanding environmental and geographical factors may promote divergent evolution and therefore the origin of new species. Until today *Megaloprepus* has been described as a single species genus. However, as a tree hole breeder of primary rain forests strong adaptation to a narrow ecological niche all over tropical America from Mexico to Peru could favor ecologically introduced diversification. For a comprehensive hypothesis testing population genetic and phylogenetic analyses were conducted and integrated into a morphological data set on widespread sampled populations as well as on Museum material. Analysis of four molecular sequence marker resulted in high population genetic structuring accompanied by nearly no gene flow between populations. Significant differences in morphology were observed in size, wing shape and coloration, as well as the structure of the male genital ligula. These differences agree with the phylogeographic clustering. Furthermore, the same groups were obtained in a phylogeny based morphological and molecular data. As a consequence, we suggest that the genus *Megaloprepus* consists of a complex of four species rather than a single species. In 1860 two previously described, later discarded subspecies should consequently receive full species status. Beside the nominal species *M. caerulatus*, *M. brevistigma* and *M. latipennis* occur with a log geographic distance in South America and Mexico, respectively. A new subspecies *M. caerulatus* subsp.nov. was discovered in Central America. These findings highly support a recent diversification in this genus probably due to a highly diverse landscape and habitat interruptions over an evolutionary time scale. Furthermore, phylogeography of the damselfly genus *Megaloprepus* may provide insights into factors driving diversification in Neotropical primary forests in general.

**OP-EB-02**

**A major evolutionary transition of egg-laying strategy in stick and leaf insects: first record of a phasmatodean ootheca**

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The eggs of stick and leaf insects (Phasmatodea) bear close resemblance to plant seeds. Adult females continuously disperse single eggs by dropping them to the litter, inserting them into crevices or attach them to twigs and leaves. Here we report a novel egg-deposition mode for Phasmatodea performed by a Vietnamese species that produces a complex egg case (ootheca) containing approximately 30 eggs in a highly ordered arrangement. The eggs are oriented radially with their anterior end at which the offspring emerges directed to the ootheca’s surface. This novel egg-deposition mode constitutes a major transition in reproductive strategy among stick and leaf insects, viz. a shift from dispersal of individual eggs to egg clumping. The adaptive advantages of ootheca formation on arboreal substrate are likely related to protection against parasitoids, e.g. chrysidid wasps, and allocation of specific host plants. This ootheca-producing species is identified as an undescribed member of the enigmatic and species-poor stick insect subfamily Korinninae. Our phylogenetic analysis of nuclear (28S, H3) and mitochondrial (COI, COII) genes recovered Korinninae as a subordinate taxon among the species-rich Necrosciinae and thus suggests that placement of single eggs on host plants might be the evolutionary precursor of ootheca formation.
OP-EB-03

Bears in a forest of gene trees: Phylogenetic inference is complicated by incomplete lineage sorting and gene flow

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Ursine bears are a mammalian subfamily comprising of six morphologically and ecologically distinct extant species. Previous phylogenetic analyses of concatenated nuclear genes could not resolve all relationships among bears, and appeared to conflict with the mitochondrial phylogeny. However, evolutionary processes like incomplete lineage sorting and introgression can cause gene tree discordance and complicate phylogenetic inferences. We generated a high-resolution data set of autosomal introns from several individuals per species and of Y-chromosomal markers. Incorporating intraspecific variability in coalescence-based phylogenetic and gene flow estimation approaches, we traced the genealogical history of individual alleles. We found considerable heterogeneity among nuclear loci and discordance between nuclear and mitochondrial phylogenies. A species tree with divergence time estimates indicated that ursine bears diversified within less than two million years. Consistent with a complex branching order within a clade of Asian bear species, we identified gene flow from Asian black into sloth bears. Moreover, gene flow detected from brown into American black bears can explain the conflicting placement of the American black bear in mitochondrial and nuclear phylogenies. Our results highlight that both incomplete lineage sorting and introgression are prominent evolutionary forces. Complex evolutionary patterns are not adequately captured by bifurcating trees, and can only be fully understood when analyzing multiple independently inherited loci in a coalescence framework. Phylogenetic incongruence among gene trees hence needs to be recognized as a biologically meaningful signal.

OP-EB-04

The Signature of Zoochorous Dispersal Dispersal Ecology and Population Genetics of Reed Beetles (Genus: Macroplea)

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Zoochorous (animal-mediated) dispersal by waterbirds is of great importance for numerous aquatic invertebrate taxa but has rarely been considered as a possible dispersal mechanism for aquatic insects. We present first evidence for bird-mediated dispersal of an aquatic leaf beetle. The fully aquatic leaf beetle Macroplea mutica shows a remarkably wide distribution throughout the whole Palaearctic, despite very little potential for active dispersal. Since host plants of M. mutica are a preferred food of many water bird species, different life stages can be ingested and dispersed by birds as a consequence.

We tested whether population genetic structure in M. mutica reflects abundances and movements of a potential vector species (therefore suggesting zoochorous dispersal). Population genetic data for 21 European M. mutica populations was mapped against data for more than 260,000 sightings of ringed mute swans (Cygnus olor). High densities of mute swans could be shown to correlate with reduced genetic differentiation between beetle (sub-) populations and with a reduced signature of geographic distance on genetic differentiation. Furthermore, M. mutica eggs could be shown to survive gut passage in mallards (Anas platyrhynchos) during feeding trials, proving potential for internal transport (endo-zoochorous dispersal), hitherto largely unknown for aquatic insects.

The study’s results strongly suggest significance of waterbird-mediated transport for dispersal of Macroplea mutica, highlighting the possible importance of endo-zoochory for aquatic organisms previously not associated with this mode of dispersal.
**OP-EB-05**

**Phylogeography of the *Hyles euphorbiae* complex (Lepidoptera, Sphingidae) - Diverse molecular patterns of introgression and evidence for hybrid zone movements influenced by climate change**

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The *Hyles euphorbiae* complex is a paramount system to study the potential impact of climate change on phylogeographic evolutionary processes. Larval and adult colour patterns suggest, that it is composed of two main lineages which are distributed in quite different climates, namely the Asio-European *H. euphorbiae* and the Afro-Macaronesian *H. tithymali*, with potential hybrid populations in several large areas of the Mediterranean. Our analyses of mtDNA sequences (2300bp COI/II) and 12 microsatellite loci of about 900 specimens from the entire distribution range support the split into two main entities and revealed that phylogeographic patterns of the potential hybrid populations ranged from no traces of *tithymali* in Galicia via admixture in both markers on Malta to strong mito-nuclear discordance of *tithymali*-derived mtDNA with *euphorbiae*-related nuclear markers in Southern Italy and on Aegean Islands. While genetic exchange across the Strait of Gibraltar is comparably limited, clinal allele distribution patterns from Canary Islands across the Maghreb to Central Italy and/or Aegean Islands (an area congruent with the range of *tithymali*-derived mtDNA haplotypes) indicate movements of this hybrid zone. This hypothesis is supported by a previous study on museum specimens which revealed past admixture of *euphorbiae*- and *tithymali*-related mtDNA haplotypes in Southern Italy and a steady increase of the latter to their current prevalence in correlation with climate warming during the 20th Century. Interestingly, this is in direct contrast to the general pattern of mitochondrial capture during introgression suggesting that mitochondrial variants might not be selectively neutral but *tithymali* is more competitive in a warming climate.

**OP-EB-06**

**Comparative phylogeography of African rhinoceros species suggests vastly different evolutionary histories**

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The two African rhinoceros species are among the most endangered mammals in the world, yet their evolutionary histories are still largely unknown. Since both species were largely eradicated from their former ranges, intensive historical sampling in museums was conducted to fill the present-day gaps in species distribution. Results suggest that geographic barriers such as rivers and tectonic rifts negatively affect gene flow in both species, but apart from this similarity, their evolutionary histories appear vastly different. Historically, genetic variation in the black rhinoceros (*Diceros bicornis*) was high, yet the devastating population collapses at the end of the 20th century affected mtDNA variation more severely than microsatellites. Of the nine evolutionary significant units identified for the black rhinoceros, seven could still remain, but the existence of two of these is highly unlikely. In contrast, genetic variation in the white rhinoceros (*Ceratotherium simum*) was partitioned into two discrete populations, each with very low variation which, most surprisingly, was not higher historically, implying that extant populations are still recovering from a relatively recent prehistoric demographic collapse. Thus, unlike the browsing black rhinoceros, whose high diversity and structure suggests that its populations remained unaffected throughout Plio-Pleistocene paleoclimatic fluctuations, the grazing white rhinoceros underwent several population crashes when grasslands contracted during interglacial periods. Even during the last glacial maximum, when the grassland biome was continuous between southern and eastern Africa, the two white rhinoceros populations appear not to have come into secondary contact.
Evolution of Electric Organ Discharge (EOD) in African weakly electric fishes: Genomics and behavioral ecology of a magic trait

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The mormyrids comprise a species-rich group of African weakly-electric fish which has undergone an enormous radiation. This is particularly true for the genus Campylomormyrus which consists of about 15 closely related species mostly restricted to the Congo basin. In a combined molecular, electrophysiological, and behavioural study, we demonstrate that (1) cryptic species are hidden behind morphologically similar (but electrophysiologically divergent) morphotypes, (2) divergence in Electric Organ Discharge (EOD) is associated with small, but significant morphological changes regarding the feeding apparatus, and (3) EOD is the trigger of species/mate recognition. Transcriptome analysis revealed differential expression between functional muscles and the muscle-derived electric organ in numerous genes. In particular, ion channel genes show signals of positive selection and divergent evolution, not only between electric and non-electric fish, but also among closely related Campylomormyrus species. The EOD may constitute a “magic trait”, i.e., a variable heritable trait which allows both for ecological diversification and species/mate recognition. Recently, we succeeded in cross-species hybridization of closely related, but differentially discharging species. These hybrids will enable us to functionally relate variation in the genome and/or transcription pattern to the relevant phenotypic traits, i.e., the EOD and the feeding apparatus. In addition, behavioural analyses will provide insights into the heritability of EOD preferences with regard to species/mate recognition.

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OP-EB-08

Regulatory gene networks that shape the development of adaptive phenotypic plasticity in a cichlid fish

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Despite its evolutionary and ecological importance, the developmental regulatory networks underlying developmental plasticity in non-model organisms remain largely uncharacterised. The cichlid fish, Astatoreochromis alluaudi is ancestral to the Lake Victoria and Malawi rapid radiation. It exhibits pronounced plasticity in response to a mechanically stimulating diet in the pharyngeal jaw apparatus, a key innovation that is believed to have promoted the spectacular diversification observed amongst East African cichlids. We gained insight into the regulatory basis of this plasticity by examining developmental expression of 19 previously identified ‘plasticity genes’ in specimens that were raised for between one and eight months on either a hard or soft diet (whole snails or finely pulverized snails, respectively). Plastic morphologies were first detected after three to five months of treatment. Interestingly, differential expression of our candidate genes preceded the onset of observable morphological divergence, suggesting that their expression contributed to the initiation of the plastic phenotypes. Strikingly, co-expression was observed amongst our candidates that belong to similar functional classes, prompting us to conduct a transcription factor binding site analysis to investigate the regulatory basis of this pattern. Interestingly, differential expression of our candidate genes preceded the onset of observable morphological divergence, suggesting that their expression contributed to the initiation of the plastic phenotypes. Strikingly, co-expression was observed amongst our candidates that belong to similar functional classes, prompting us to conduct a transcription factor binding site analysis to investigate the regulatory basis of this pattern. Our results allowed us to construct a regulatory network that putatively contributed to the observed plasticity, which is controlled, in part, by the mechanically responsive transcription factor AP1. By investigating phenotypic plasticity throughout a developmental time-course we have identified an environmentally responsive, interconnected regulatory network that contributes to the development of the integrated plastic LPJ phenotypes in a recently established molecular model for adaptive plasticity.
OP-EB-09

Variation in adaptive resilience in natural populations of an ecologically important freshwater fish species

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Habitat modification is accelerating throughout the range of many freshwater species. The pace and extent of this change raises questions about how populations may adapt to new environmental conditions. Although the theory on adaptation is well developed, we lack knowledge about the genomic basis of population adaptation and divergence. An understanding of the evolutionary potential of species to adapt to rapid global change is vital for the identification of vulnerable populations and hence to improve conservation strategies. Here we examined genetic variation at over 17000 SNP loci across the landscape for populations of the crimson spotted rainbowfish (Melanotaenia duboulayi). We searched for correlations between the genomic data and relevant environmental variables while accounting for spatial structure within the dataset. Our analyses show clear evidence for selection acting consistently on particular loci in similar habitat types. Selected loci are related to genes with ontologies known to be important for metabolism and cell signaling. Furthermore, consistent with expectations from the climate variability hypothesis (CVH), populations from the north of the species range showed more restricted levels of adaptation compared to those in the southern more climatically variable parts of the range. This suggests that as the climate warms, populations from northern parts will be more vulnerable to local extinction due to a lack of adaptive genetic variation. Researchers can use our approach to predict how other species may respond to a rapidly changing environment.

OP-EB-10

Chemical warfare no solution against parasite attack: Tribolium castaneum vs. Beauveria bassiana

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Question:
The beetle Tribolium castaneum disposes an extended external immune defence, consisting of the secretion of quinones into its environment in addition to the classical invertebrate immune defence, with broad antimicrobial properties.

Here, we wanted to gain a better understanding of if and how the external defence of T. castaneum responds upon actual contact with a parasite and how the two interact.

Methods:
We conducted a coevolution experiment with the entomopathogenic fungus Beauveria bassiana. Throughout the experiment we measured levels of internal and external immune traits in the host. At the end of the coevolution also survival was taken as a proxy of resistance. In parallel, we also took the parasite’s perspective by testing for virulence towards the host as well as for potential resistance against the host’s external secretion.

Results:
No change in host external immunity or survival was observed as a consequence of host-parasite coevolution, however, PO responses in evolved beetles showed increased flexibility. While this host response is more cryptic, we were able to show that B. bassiana increased in virulence during the course of coevolution, and that this was a result of the fungal isolates evolving resistance to the external immune defences of the T. castaneum beetles.

Conclusions:
The cryptic changes found in T. castaneum response to coevolution with B. bassiana are in line with other coevolution experiments and various hosts. However, non of these also took the host perspective, where our findings present a rare example of an experimentally coevolved increase in virulence, where the exact barrier of host immune defence overcome by the parasite has been described, namely the external secretion of quinones.
OP-EB-11

Infection and immune priming in *Tribolium castaneum* - a transcriptomic approach

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Host-parasite coevolution is characterised by fast evolutionary changes, based on genetic adaptations in hosts and parasites or pathogens. Moreover, hosts are able to combat parasites with the help of a phenotypically highly plastic immune system that can come with specific memory. There is accumulating evidence for a memory-like phenomenon in the immune defence of invertebrates. Such ‘immune priming’ can even be rather specific. Invertebrates do not possess the machinery of the vertebrate adaptive immune system, and the mechanistic underpinnings of immune priming are still largely unknown. In the red flour beetle *Tribolium castaneum*, immune priming for resistance against the entomopathogen *Bacillus thuringiensis* has been demonstrated. Immune priming arose after septic ‘pricking’ as well as oral pathogen exposure. Here, a transcriptomic approach will be presented that aims at deepening our understanding of the immunological consequences of these different ways of infection and of the evolutionary relevance and mechanistic underpinnings of immune priming.

OP-EB-12

Parasite-induced changes in host behavior and gene expression after infection

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Parasite infections may affect host organisms on several levels from physiology, morphology, life history to behavior, and thus may have great impact on the hosts’ fitness. The observed changes in the host can either be attributed to host defenses to reduce the costs of infection, they can be by-products of infection, or they can be the result of manipulation by the parasite in order to increase its transmission or reproduction. In social insects not only the infected individual itself but the whole colony is affected by the parasites presence and has to buffer the consequences as community. We could show that the cestode parasite *Anomotaenia brevis* greatly affects the behavior, life history and morphology of individual *Temnothorax nylanderi* host ants. In addition parasitized colonies show lower aggression levels than healthy colonies, or colonies after removal of infected individuals. To understand the proximate mechanisms of parasite-induced behavioral changes, brain gene expression patterns between infected individuals, their healthy sisters and individuals from uninfected colonies were investigated. We will report on the functional gene categories overrepresented in parasitized individuals compared to the others and specifically investigate genes known to mediate aggressive behavior and immunity in insects.
Evolutionary Biology

OP-EB-13

Social immunity in earwigs: Frass production provides antimicrobial protections in an insect with maternal care

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The evolution of social life requires mechanisms limiting the risks of disease transmission between group members. Whereas these mechanisms are well known in eusocial insects (e.g. ants, bees and termites), it remains unclear whether they also emerged in species with less derived forms of social life, such as family life. Here, we investigated whether frass production limits the risks of disease transmission by inhibiting microbial growth in nests of the European earwig, an insect species in which females provide extended forms of care to their offspring. To this end, earwig frass pellets were collected and used in radial diffusion assays to test whether 1) frass produced by mothers and offspring inhibit microbial growth, 2) such inhibitions differ between the two types of frass and finally whether 3) mother-offspring interactions trigger such inhibitions. Overall, our results demonstrated that earwig frass inhibited the growth of GRAM+ bacteria (Staphylococcus aures), mold (Aspergillus niger) and yeast (Saccharomyces cerevisiae), but not GRAM- bacteria (Escherichia coli). Maternal frass entailed higher levels of inhibition against S. aures than offspring frass, whereas the opposite pattern was found against A. niger. Interestingly, mother-offspring interactions canceled female frass inhibition against S. cerevisiae, but had no effect on the one by offspring frass. Overall, these results reveal that frass production is a form of social immunity in earwigs, which provides important benefits to family members by limiting microbial growth within the nest.

OP-EB-14

Causes of intraspecific variability in associative learning in the parasitic wasp Nasonia vitripennis

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Within the animal kingdom the ability to learn is a key to behavioral adaptation to changing environments. Yet, learning behavior varies cross species. As a prerequisite for the evolution of species-specific or even population-specific learning behavior considerable genetic and phenotypic variability within that trait needs to be present within species. While several species of parasitoid wasps have been shown to have the ability to learn associating chemical cues with the presence of hosts, intraspecific variability in this learning behavior has received little attention. We tested memory retention in the parasitic wasp Nasonia vitripennis (Walker) (Hymenoptera: Pteromalidae) using a genetically diverse strain and an iso-female line , bearing a low genetic variability. Our findings suggest that the two strains differ in memory formation from each other and also from yet another strain of N. vitripennis used in previous studies. The genetically diverse strain in our study showed much more variability in memory retention than did the iso-female line, and this variability decreased with increasing cue reliability. Hence, we show that learning of host associated cues in N. vitripennis shows considerable variability, which is at least partly genetic and also varies with cue reliability. Even though the associative learning of host cues is most likely under strong natural selection in parasitoid females, genetic variability is maintained within the species.
OP-EB-15

Allele recognition drives mate choice in a parasitic wasp

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The gregarious ectoparasitoid *Bracon brevicornis* (Wesmael) has complementary sex determination (CSD). Especially under inbreeding conditions, CSD leads to the production of diploid males at the expense of fertile daughters. Diploid males in this species are able to sire triploid daughters at a low rate, which only occasionally produce offspring. Avoidance of sib mating should reduce costly diploid male production and has been found in several Hymenoptera with CSD. In *B. brevicornis* however, we observed that brothers were not as consequently rejected as we had expected from the negative consequences of matched matings. This could be explained by an extraordinarily long mating latency that facilitates pre-mating dispersal, lowering the chance that receptive females are courted by a brother. On the other hand, we found a relatively high rejection rate of males that were not closely related to the female but still originated from the same population. We tested if females would mate disassortative according to their own sex determining alleles, similar to mice and men and MHC. Indeed, allelic recognition can explain the observed pattern quite well: females that were forced to mate with an initially rejected male produced significantly more diploid male offspring than females that mated an initially accepted partner. We thus conclude that mating partner rejection is at least partly due to allelic incompatibility in *B. brevicornis*. Such a mechanism could contribute to the maintenance of allelic diversity in this species, even in bottlenecked populations.

OP-EB-16

Evolution of homomorphic sex chromosomes in anurans: Testing predictions from Haldane’s rule on introgression patterns in secondary hybrid zones using transcriptome-based markers

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Haldane’s rule, which states that in interspecies crosses, the heterogametic sex (XY-males, ZW-females) suffers greater potential fitness depressions than the homogametic sex (XX, ZZ), is one of the most universally applicable ‘laws’ in evolutionary biology. For XY-systems, this implies that under secondary contact (after allopatric speciation), autosomal introgression is greater than X-chromosomal and mitochondrial, while both should surpass Y-linked introgression. However, in animals with homomorphic (microscopically indistinguishable), occasionally recombining sex chromosomes, this rule remains poorly studied, so in amphibians. Applying classical microsatellites to genetic families and backcrossed non-model frogs, we identified sex-linked, although anonymous markers. To bridge this gap, we tested sex linkage of sequence repeats in known genes from individual transcriptomes. Despite >200 My of divergence, synteny between the only well-assembled anuran genome (*Xenopus tropicalis*) and non-model species (Hyliidae, Bufonidae) identified almost the entire (here autosomal) *X. tropicalis*-linkage group 1 to be homologous to their sex chromosome. This allowed developing additional markers from adjacent chromosomal blocks. We have done this for four lineages of Palearctic green toads (*Bufo viridis* subgroup) that arose at different times and form secondary contact zones, previously studied at autosomal loci. Comparisons of introgression patterns involve multiple types of sex-linked markers (classical & transcriptome-based msats, introns, SNPs, mtDNA). Tests are underway to understand if differential autosomal vs. sex-chromosomal introgression in these hybrid zones occurs, and if predictions from Haldane’s rule apply.
OP-EB-17

The evolution of social monogamy in primates: Insights from the lemurs of Madagascar

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The study of the evolution of mammalian monogamy continues to generate much controversy. Recent comparative studies reached contradictory conclusions about the evolutionary origins of social monogamy, but they ignored variation in social bond quality between pair-partners. Compared to monkeys and apes, the lemurs of Madagascar represent an ancestral primate radiation with many pair-living species exhibiting striking variation in pair cohesion. I therefore review recent field studies of lemurs with variable intersexual bonds and use phylogenetic reconstructions of lemur social organization to reconstruct major steps in their social evolution. These analyses revealed two types of pair-living and suggested four cumulative steps in evolutionary transitions from a solitary life towards pair-living among the Cheirogaleidae and Lepilemuridae, whereas the origins of monogamy among the Indriidae remain unresolved for the time being. The Lemuridae represent the only group of mammals where pair-living evolved from group-living ancestors, and I examine inter- and intra-specific variation in group size to explore the possibility that female competition may have driven this transition. The present analyses suggest that ecological factors may have initially facilitated the formation of dispersed pairs from solitary ancestors, and that benefits of infanticide avoidance may have been of more immediate importance in transitions from group-living ancestors to associated pairs, thereby offering an explanation for the contradictory results of previous comparative studies.

OP-EB-18

Cost and benefit dependent migrating strategies in male crested macaques (Macaca nigra)

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Dispersal and immigration into a new social group is often accompanied by substantial costs for animals due to risks of predation, starvation and resistance from residents. Costs may, however, vary between individuals depending on the individual’s characteristics and the target group constellation. Whether or not dispersing individuals use different strategies according to the costs of migration and the expected reproductive benefits in the new group is unknown. We therefore aimed to investigate migrating decisions in crested macaques according to costs and expectable benefits, a primate species with a high reproductive skew and a high rate of male migrations. Data on three groups of wild crested macaques were collected from 2006 to 2012 in Tangkoko, North Sulawesi, Indonesia. We found immigration events to be clumped with some males migrating into new groups first (leaders) followed by others (followers) that immigrated when the group already faced hierarchical instabilities due to the leader. Leaders experienced significantly more often injuries, i.e. costs, but also generally achieved high rank. Followers, in contrast, reached mostly low rank. Natal males immigrated into a new group as leaders, usually achieving alpha position, and seemed to delay dispersal until reaching sufficient body condition. We conclude that male crested macaques assess their body condition and either use a high-cost high-benefit strategy by immigrating as leaders, or a low-risk low-cost strategy by immigrating as followers. Our study shows that individuals adjust their migration decisions to expectable costs and benefits based on their individual abilities.
OP-EB-19

The evolution of cuticular hydrocarbon profiles in ants

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Insect cuticles are covered with cuticular hydrocarbons (CHC), which serve two main functions: waterproofing and information transfer. The CHC profile of an ant encodes information on its species identity, colony, caste, sex, etc. Recently, our knowledge on causes and functions of intraspecific CHC variation has advanced drastically. However, little is known to date about the evolutionary causes of the strong variation between species. In particular, phylogenetic or physiological constraints, but also selection pressures on CHC profiles are poorly understood.

We analysed cuticular profiles of 39 *Camponotus* and 46 *Crematogaster* taxa from around the world, searching for phylogenetic and physiological constraints and for selection pressures on CHC composition. CHC profiles did not appear to experience strong phylogenetic constraints. Even sister species exhibited completely different profiles. However, we identified physiological constraints. First, dimethyl alkanes and alkenes rarely co-occurred in the same species. Second, hydrocarbon chain length was related to the substance class composition of a CHC profile, which might be caused by the need to maintain a specific viscosity of the epicuticular layer. Finally, climate strongly influenced substance class composition. Tropical rainforest species had more unsaturated compounds than species from more arid habitats. We explain this by variance in draught stress, as less waterproofing is required in humid tropical rainforests. The length of the hydrocarbon chains was unaffected by climate, but was higher among ants in interspecific associations. We conclude that CHC profiles can evolve quickly, but experience constraints posed by climatic conditions, interspecific interactions, and CHC viscosity.

OP-EB-20

Complexity of the olfactory system across and within Hymenoptera - *Atta vollenweideri* as a case study

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In eusocial insects, odors play an important role for communication and hymenopteran species possess highly developed olfactory systems. Within the first olfactory neuropile, the antennal lobe (AL), glomeruli are the functional units for odor information processing. We assume that the number of glomeruli can serve as a measure for odor processing capacity. Ancestral state reconstruction revealed that the common ancestor of Hymenoptera had a high number of glomeruli. Based on allometric relations of sensory neuropiles and higher information processing centers, we could confirm that the number of glomeruli is a well-suited measure for olfactory processing capacity, and can be used to assess the sensory bias of a species towards olfaction or vision. Within Hymenoptera, one ant clade, the highly derived Attini shows a particularly high number of glomeruli [1]. This clade is characterized by having an obligate symbiosis with a fungus they grow and feed on. The high number of glomeruli might have facilitated the evolution of such elaborate farming systems and a complex social organization. The most derived fungus-growing species are the leaf-cutting ants (*Atta* and *Acromyrmex*). In *Atta*, even within the same species the number of glomeruli differs in castes and worker subcastes (AL-phenotypes, [1]). These AL-phenotypes support the hypothesis that increased specialization can lead to a loss in neuronal processing capacity. We used the AL-phenotypes to identify receptors involved in olfactory communication [2]. Using receptor gene homologies it is now possible to gain new insights into the evolution of pheromone communication systems in Hymenoptera.

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OP-EB-21

Phoretic mites as a model for co-evolutionary dynamics and ecological speciation

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The enormous diversity of life is a striking and at the same time puzzling phenomenon. While the contribution of ecological factors to speciation have now widely been acknowledged, good model systems for ecological speciation remain rare. One ecological factor that may lead to speciation of parasites are host-specific adaptations. Here, I present data suggesting a new model for integrating co-evolution and speciation research: mites of the Poecilochirus carabi species complex.

The mites are associated with burying beetles, which are known for their extensive brood care behavior. Different mite species specialize on different beetle species, and I show that mite fitness suffers when individuals associate with another than their main host species. I present a range of mite traits that are specifically adapted to each mite species’ main host. Of these traits, one stands out: generation time. The mites use beetles for traveling between resources and reproduce at resources during the beetle brood care phase. The next mite generation needs to be fully developed before brood care is over and the parental beetles leave the resource, because parental beetles are by far the best host individuals for the mites.

Beetle brood care duration varies between populations, which holds the potential for local adaptation by the mites. I explore in how far it causes divergent evolution in mites from different populations. As fundamental live history traits such as generation time can be linked to reproductive traits such as mating behavior, generation time may be a “magic trait” causing speciation in the mites.

OP-EB-22

Patterns of ancient asexuality in oribatid mites

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The question of why sexual reproduction is maintained throughout evolutionary time despite its two-fold cost is under debate since decades. Several ecological and genetical models hypothesize why sexuals should win over asexual lineages. Asexuality is commonly regarded as evolutionary ‘dead-end’, as most asexual eukaryotes seem recent offshoots scattered throughout the tree of life. However, some eukaryotic groups have likely persisted for millions of years without sex, such as oribatid mites. This ancient taxon comprises several clades of sexual and asexual taxa with both young and old lineages, with some even considered ‘evolutionary scandals’. This unique pattern designate oribatid mites as model organisms for statistically testing hypotheses of asexual genome evolution using evolutionary replicates. In a first approach, we generated two sexual and two asexual ‘draft’ genomes from major clades to test whether old asexual lineages I) loose deleterious transposable elements, II) show base composition shifts in mitochondrial genomes due to tight linkage, III) show decay of male-specific traits and IV) avoid deleterious mutation accumulation, in order to survive over evolutionary timescales. If some lineages are able to avoid genetic meltdown, there must be a proximate ecological cause for the emergence and maintenance of sexually or asexually reproducing organisms.
OP-EB-23

Quantifying realized inbreeding in wild and captive animal populations

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Molecular measures of inbreeding that are typically used in animal populations do not measure inbreeding at the scale that is most relevant for understanding inbreeding depression - namely the percentage of the genome that is identical-by-descent (IBD). The inbreeding coefficient F obtained from pedigrees is a valuable estimator of IBD, but pedigrees are not always available, and also cannot capture inbreeding loops that reach back in time further than the pedigree. We here propose a novel molecular approach to quantify the percentage of the genome being IBD with high confidence and we apply it to a wild and a captive population of zebra finches (Taeniopygia guttata). We genotyped 260 SNPs spread over four genomic regions in each of 948 wild and 1057 captive individuals. This allowed us to confidently determine whether any of these regions was completely homozygous within an individual, which implies IBD. In the highly nomadic wild population, we did not find a single case of IBD, indicating that inbreeding must be extremely rare (95% CI for F = 0.00094). In the captive population, with a known five-generation pedigree, pedigree-based F (F_ped = 0.013) underestimated the realized inbreeding coefficient (F = 0.064), as expected given that pedigree founders were already related. We suggest that this SNP-based technique is generally useful for estimating the realized amount of inbreeding in wild and captive populations, and we show analytically that it can capture inbreeding loops that reach back up to a few hundred generations.

OP-EB-24

Genome size variation and song attractiveness in grasshoppers: sexual selection against large genomes?

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Genome size is largely uncorrelated to organismal complexity and adaptive explanations, genetic drift as well as intragenomic conflict have been proposed to explain this observation. An intragenomic conflict arises when selfish genetic elements proliferate and increase genome size at a fitness cost to the host genome. We have studied the impact of genome size on male attractiveness in the bow-winged grasshopper Chorthippus biguttulus. Grasshoppers exhibits particularly large variation in genome size due to the high prevalence of supernumerary chromosomes that are considered (mildly) selfish, as evidenced by non-Mendelian inheritance and fitness costs if present in high numbers. We ranked male grasshoppers by song characteristics that are known to affect female preferences in this species and scored genome sizes of attractive and unattractive individuals. We then test if attractive and unattractive singers differ in their genomes sizes, which would demonstrate that genome size is reflected in male courtship songs and that females select songs of males with small genomes. Such mate preference would effectively selects against further increase in genome size and could suggests an underappreciated route of how choosy females could gain indirect benefits. The data can also provide a novel example of how sexual selection can reinforce natural selection and act as an agent in an intragenomic arms race.
OP-M-IT-01

How snakes fly: effects of morphology and behavior on aerodynamics

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‘Flying’ snakes of the genus *Chrysopelea* are the only limbless animals that glide through the air. Despite a lack of limbs, these arboreal snakes take off by jumping, glide through the air without using obvious control surfaces, maneuver, and safely land without injury. The behavioral and morphological features that enabled the evolution of this remarkable suite of behaviors are poorly understood. Here, I discuss the relationship between the snake’s key morphological and behavioral features and its aerodynamics. Dorsoventral flattening and high-amplitude aerial undulation are the two most prominent features of the snake’s gliding system. Aerial undulation may serve to maximize the length of perpendicular body available for lift generation and also to position downstream body segments for wake interception. Posteriorly-directed traveling waves may additionally function to control pitch and roll instabilities associated with otherwise unfavorable asymmetrical body orientations. Upon takeoff, the snake transforms to a flattened cross-sectional shape that exhibits relatively high lift-to-drag ratios across a wide range of angles of attack. The mobility and control of rib movement necessary to maintain this shape while undulating may have originated as a specialization for grip control, relevant for life in an arboreal environment.

OP-M-IT-02

Hemichordate morphogenesis revealed by modern techniques and its implications for deuterostome evolution

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Hemichordata is a small group of marine deuterostome invertebrates. It consists of two subgroups, the sessile and small colonial pterobranchs and the solitary vermiform enteropneusts (acorn worms). In particular enteropneusts take a pivotal role concerning the evolution of deuterostomes, because of specific morphological features in addition to their phylogenetic position. Enteropneusts exhibit a number of typical chordate characters, such as pharyngeal gill slits, a hollow nerve cord, and a notochord homologue. Furthermore, they are closely related to echinoderms (sea urchin, sea star), with them they share a trimeric coelomic organization as well as a unique larval type - the dipleurula. The vast majority of histological investigations on enteropneusts have been conducted around the turn of the 20th century and provided numerous insights into the development and general morphology using classical methods. Strangely, the interest in enteropneust research has then declined within the last half of the 20th century and relatively few works were published. Only recently, with upcoming molecular genetic techniques and the new field of “EvoDevo” research, people have rediscovered the potential and importance of enteropneusts in unravelling evolutionary developmental questions. However, for any inferences concerning the evolution of morphological characters including ancestor reconstructions, detailed descriptions on the morphological level are absolutely essential. Therefore, I apply a combined methodological approach comprising SEM, TEM, serial semithin sectioning, 3D-reconstruction as well as immunocytochemistry. I will give an introduction to enteropneust morphology revealed by modern techniques and highlight import contributions to the field.
**OP-M-01**

**Multifunctional surface structures on black scales of the West African Gaboon viper (Bitis rhinoceros)**

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The West African Gaboon viper (Bitis rhinoceros) is well camouflaged in the African rain forest. Its back has a geometrical pattern of velvet black markings contrasting to the pale surfaces and thereby imitates the light and shadow spots on the ground. Our research sheds light on the functional principles of the velvet black coloration and further exceptional properties of the black scales of the snake. Scanning electron micrographs of the black scale surfaces reveal unique leaf-like microstructures striated with nanoridges. Spectroscopy, contact angle measurements, and contamination experiments provide not only evidence for the anti-reflective properties of these surface structures leading to an ultrablack appearance, but also demonstrate superhydrophobic and self-cleaning properties of the scale surfaces. This surface multifunctionality of the black spots of the scales ensures that the snake is able to maintain its high contrasting coloration also under wet and soiling conditions.

**OP-M-02**

**Epithelial crypts as accessory olfactory organs in African and South American lungfish (Dipnoi) - a comparative study**

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African lungfish (Protopteridae) are the first non-tetrapod animals where anatomically unique epithelial crypts within the nasal cavity were proclaimed as accessory olfactory organs, and interpreted as primordial vomeronasal structures. The traditional point of view sees the olfactory organ of non-tetrapods as an undivided, singular system, and the evolutionary development of the vomeronasal organ is generally dated within the emergence of tetrapods.

The present study subjected the nasal cavities and epithelial crypts of West African and South American lungfish (Protopterus annectens and Lepidosiren paradoxa) in different developmental stages to a comparative morphological study, using histology and scanning electron microscopy. In addition, immunohistochemical analyses of the respective olfactory systems were accomplished.

We were able to confirm the data already available on *Protopterus*, and also found the very same epithelial crypts being present in the nasal cavity of *Lepidosiren*. The epithelial crypt anatomy of *Protopterus* and *Lepidosiren* exhibits several similarities, like the symmetrical composition and orientation of sensory and nonsensory epithelia, and the communication of the crypt lumen with the nasal cavity through a minute pore. However, there are remarkable differences in the cell surface differentiations of the sensory crypt epithelia of the two lungfish species. Furthermore, the course of numeral increase of the crypts during ontogeny significantly differs between *Lepidosiren* and *Protopterus*. In both investigated species the sensory crypt cells show a distinct G protein subunit α2-immunoreactivity in their surface structures, indicating the involvement of this G protein in the signal transduction pathway, a typical feature of vomeronasal neurons.
OP-M-03

Conformity or Disparity: Size and shape of canine lower cervical vertebrae

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Despite the limited number of cervical vertebrae, former studies suppose a functional tripartite regionalisation of the mammalian neck skeleton similar to that of other amniotes. In contrast, anatomists usually divide the cervical spine (CS) only into two portions: the upper CS (Atlas, Axis) and the lower CS (C3-C7). Although some differences between the lower cervical vertebrae are known, they are often labelled as very similar. To broaden our understanding of the structure of the CS, we examined the shape differences between the lower cervical vertebrae of dogs as a model organism for mammalian morphological plasticity. A broad diversity of breeds was analyzed within the 3D geometric morphometric approach basing on medical CT scans.

Principal component analysis reveals the vertebral level and the breed size to be major factors determining vertebral shape. However, both factors operate independently. Thus, shape change along the lower CS is the same for small as well as for large breeds. On the other side, shape change from small to large breeds is the same for every vertebral level. Significant disparity between all vertebrae is confirmed by canonical variate analysis and MANOVA. Additionally, vertebral body and arch exhibit strong covariation along the vertebral level highlighting the role of vertebral position within the CS for predicting its morphology. Nevertheless, only C6 and C7 are assignable unambiguously within cluster analyses revealing their unique morphology. Hence, morphology reflects the functional tripartition of the CS into the specialised upper CS (Atlas, Axis), the more uniform middle CS (C3-C5), and the specialized lower CS (C6, C7).

OP-M-04

Lost variation - The hyoid bone in rodents

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The hyoid in mammals is a small bone derived from a branchial arch and consisting of several elements. Due to its tiny size and the fact that it is as a rule connected to the skull only by soft tissue it usually gets lost during preparation, particularly in small animals like rodents. Hence, very little information concerning its variability in small mammals is available. We analysed a data set of 3D microCT scans of 121 hyoid bones from 65 rodent species, including amongst others mice, gerbils, guinea pigs, and squirrels. Our analyses showed tremendous differences in size, shape, position, and number of bony elements. Major shape changes have occurred mostly between distantly related groups with gradual reductions from squirrels to mice, but also between and even within some species a remarkable degree of variation can be found, which is presently investigated.
**OP-M-05**

**Sinus hair sensing in forelimb positional control during the locomotion of rats (Rattus norvegicus, Rodentia)**

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Facial (mystacial) vibrissae, a kind of sinus hairs within the group of tactile hairs, have been intensively studied for muscle activities, neurological patterns, anatomy details and their biological role in different species. The skill to move in a more or less regular pattern (whisking) is known from various species, like rats. In addition to these whiskers rats have other sinus hairs, e.g. the non-moving carpal ones on the forelimbs. It has been assumed that carpal sinus hairs have a sensory function during locomotion on different substrates by detecting discontinuities while whiskers detect vertical obstacles. The possibility of a coupled sensorimotor control between the limbs and the sinus hairs might lead to a stabilized locomotion over uneven substrate. To test this hypothesis several spatiotemporal speed dependent parameters as well as kinematic data for the limbs were quantified and linked to the motion of carpal and facial sensors. Parameters were measured from x-ray and high-speed videos. A treadmill with continuous and discontinuous substrate was used. Rats had to walk under the presence and absence of the carpal and/or mystacial tactile hairs. Data were collected for a speed range of 0.2 to 0.5 m/s. Collecting tactile information by whiskers and carpal sinus hairs during touchdown and swing phase of the limbs is an important factor to get information about the substrate where the limb is going to be placed next. Loss of the sinus hairs affected the degree of parameter variation but not average parameters or the failure rate of the limbs during walking on the perforated treadmill. The motion of whiskers is affected by the presence/absence of the carpal sinus hairs and might compensate the loss of this substrate sensor.

**OP-M-06**

**A functional morphological study of bypass elements in Tethya wilhelma using finite element modelling and particle tracking velocimetry.**

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In the classical model of flow for sponges the aquiferous system consists of choanocyte chambers, incurrent- and excurrent canal system elements. However, morphological studies in the last years revealed yet another architectural canal system structure, the so called bypass elements. These structures lead to a connection between the incurrent and excurrent canal system without passing choanocyte chambers. Therefore alternative flow pathways are likely to occur, e.g. recirculation of water. In order to study the fluid mechanics impact of bypass elements in *Tethya wilhelma* we investigated internal flow fields and the canal system architecture. A virtual 3D canal system model was developed based on SRµCT data sets. This model was utilized in FEM flow simulations. In vivo particle tracking velocimetry measurements of oscular outflow served as initial boundary conditions. SEM investigations supplemented detailed morphometric information on the cellular level for various elements of the aquiferous system. Our simulation results yield no evidence for recirculation of water within the canal system. General flow regimes for water passing through the interconnecting bypasses proceed from incurrent lacunae directly into the atrium. Flow velocities in these regions are not distinct higher than in canals with comparable diameters. Based on resistance theory and fluid dynamic properties of flow there is a correlation between the span of bypasses and the response of a hierarchical fluid transport network. In the case of the *T. wilhelma* canal system bypasses lead to an increased perfusion of body parts with bypass elements. Bypass elements might therefore be an element of a more complex flow regulation system in leucon-type canal systems.
OP-M-07

**Regeneration and reproduction in Syllidae (Annelida)**

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Annelids possess extensive regenerative abilities. Basically, three cases could be distinguished: Regeneration after traumatic injury, regeneration during asexual reproduction and regeneration during sexual reproduction. The first two processes are widely spread within Annelida; however, the latter process, called schizogamy, is restricted to a few marine groups, such as the Syllidae. In schizogamy, sexually mature individuals produce sexual units called stolons in their posterior ends. After their detachment, the adult regenerates the posterior end. Within Syllidae, two groups are able to reproduce by schizogamy (Syllinae and Autolytinae), but the process shows differences in each of them.

We provide herein a detailed study of the stolonization and regeneration process in *Typosyllis* sp. (Syllinae), complemented by a comparison with the stolonization in *Myrianida prolifera* (Autolytinae). The regeneration of the anterior and posterior body ends of *Typosyllis* sp. after traumatic injury, as well as different stages of stolonization in both species were studied using immunohistochemical staining techniques (F-actin, alpha-tubulin) combined with subsequent cLSM.

Our data reveal an anterior regeneration of the prostomium and up to three segments in *Typosyllis* sp., but most parts of the ectodermal foregut, including the proventricle, are not re-developed. In contrast, posterior regeneration appears to be complete. During stolonization, *Typosyllis* sp. develops just one stolon at once, while *M. prolifera* develops a chain of stolons.

These findings help to understand the relationship of regeneration and stolonization within Syllidae, as well as the relationship of regeneration and reproduction within Annelida in general.

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OP-M-08

**Functional morphology of adhesive tongues in frogs**

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Frogs are well known to capture elusive prey items by using a sticky tongue that can be flipped out of the mouth. Frog tongues thus provide an interesting example of a biological wet adhesive system. Remarkably, frog tongues operate at very high speeds and thus very short time frames to actually establish a contact. Further, they stick to a wide range of different surfaces, e.g. insect cuticle, fur, feathers. However, little is known about the actual mechanism that causes adhesion of frog tongues. Here we recorded tongue forces in South American horned frogs (genus *Ceratophrys*) against a glass surface. We found that these frogs can generate pulling forces with their tongues that were on average 1.4 times the body weight of the animals. We assume that the actual mechanism of adhesion in frogs relies on the presence of a liquid (mucus) and the surface structure of the tongue. The surface structure possibly has an effect on the size of the contact area, the utilisation of capillary/viscous forces, and the friction of the tongue against a prey surface. Micro-CT imaging and scanning electron microscopy (SEM) reveal a high degree of morphological variation among the tongues in different species. This variation can be observed on multiple scales from the macroscopic shape of the tongue towards the microscopic organisation of the tongue surface. Several species show hair-like papillar outgrowths of the tongue epithelium, in other species, however, the tongue surface is more or less smooth. Further, in species with papillar outgrowths, these papillae vary notably in their hierarchical organisation as well as their aspect-ratio. Functional and phylogenetic patterns of these variable surface morphologies remain to be explored.
**OP-M-09**

**Comparative development of the body wall musculature in Nemertea provides insights into the evolution of body wall musculature in Spiralia**

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Nemertea is a spiralian clade comprising approximately 1300 species of mostly marine, nocturnal predators. They are without doubt monophyletic due to a peculiar apomorphic character represented by a dorsally located, eversible proboscis that is housed in a fluid filled secondary body cavity, the rhynchocoel. The proboscis opens at the anterior tip of the animal via a tube termed rhynchodaeum. Additional derived characters evolved in the stem lineage of Nemertea include a ring-shaped brain surrounding the rhynchodaeum instead of the pharynx and endothelialized blood vessels. Within Spiralia, recent data suggest a close phylogenetic affinity of Nemertea to Trochozoa although the sister group of Nemertea is currently still a matter of debate. This is mainly due to Nemertea exhibiting character states that are arguably ancestral in Trochozoa such as a multiciliated epidermis, a medullary cord-like nervous system, the lack of segmentation, and a continuous body wall musculature comprising minimally two layers. They comprise an outer circular and an inner longitudinally arranged sheath. Within various sub-clades of Nemertea, additional layers may occur. During development most species pass through larval stages that swim with the aid of the epidermal cilia. To elucidate the ancestral pattern of arrangement and mode of development of the body wall musculature developmental stages of all major nemertean sub-clades have been studied comparatively by means of fluorescent staining and confocal laser scanning microscopy. The results obtained allow for a hypothesis to be stated regarding the ancestral design and mode of development of the body wall musculature in Nemertea, Trochozoa, and Spiralia.

**OP-M-10**

**The ground pattern of larval brachiopod eyes revealed by 3D-reconstruction**

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Although eyes belong to the most fascinating organs of metazoan animals and have been studied since decades, the evolution of eyes is still far from being understood. Recent findings of ciliary photoreceptors within the larval eyes of brachiopods provoke further discussion about the evolution of eyes within Bilateria. Due to the findings of this ciliary photoreceptors within protostomes, and on the other hand rhabdomeric photoreceptors within deuterostomes, the hypothesis that there is a clear split of ciliary and rhabdomeric photoreceptors within Bilateria has to be rejected and further information is needed to obtain an idea of how eyes evolved.

Many larvae of marine invertebrates are known to have the most simple form of an eye, formed of just two cells, one acting as the photoreceptor cell and the other one as the shading pigment cell. This is also known as the prototype of an eye.

Brachiopod larval eyes are as well build of just two cells, but have a unique setup compared to all other Lophotrochozoa since they are equipped with two photoreceptor cells both with a second function, one forming the lens, the other one forming a pigmentcup. We investigated the larval eyes of specimens representing four major groups of living brachiopods, i.e. Thecideoidea, Laqueoidea, Kraussinoidea and Megathyridoidea, with ultrastructural and immunohistochemical methods. The results of our investigations strongly indicate that the ancestor of articulate brachiopods had larval eyes formed of two light-sensitive cells using ciliary photoreceptors for vision. The eyes must have been reduced in those taxa with eyeless larvae, even if some of them are still able to react to light.

Here we present 3D-models of the investigated eyes and hypothesize a ground pattern for this unusual larval eyes in brachiopods. This data can be used to compare the architecture of larval brachiopod eyes with other bilaterian groups and will additionally help to understand the evolution of eyes within Bilateria.
OP-M-11

Can the neuronal innervation of sensory organs support evolutionary loss of hearing organs in the cave cricket (Ensifera: Rhaphidophoridae: Troglophilus neglectus)?

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Among Ensifera, tympanal hearing organs occur in the foreleg tibia of crickets (Gryllidae) and bushcrickets (Tettigoniidae). The homology of ears between these groups is not resolved. The reduction of hearing organs in all non-hearing (atympanate) ensiferan taxa was recently inferred from molecular phylogeny. Remarkably, elaborate sensory organs clearly homologous to the tettigoniid auditory sensilla occur in the majority of atympanate Ensifera. Therefore, we studied the sensory organs in a species of the atympanate cave cricket (Rhaphidophoridae), Troglophilus neglectus, testing for sensilla possibly also homologous to auditory sensilla. The neuronal innervation of sensory organs in the tibia was documented by axonal tracing.

The innervation confirms two sensory organs, the subgenual and the intermediate organ, which are also known in tettigoniid species. Both are in most cases innervated by a single nerve. Some variation occurs in the innervation of both the subgenual and the intermediate organ, to similar proportions. Sensilla homologous to the auditory organ are not clearly evident.

Obviously, the entire set of auditory sensilla might have been reduced during evolution. However, this would require different rates of reduction (tympana or neuronal elements) across Ensifera, while neuronal structures are generally rather conserved in evolution. The innervation pattern is more similar to the innervation patterns known for sensory organs from outgroup species of locusts (Caelifera) and stick insects (Phasmatodea). These findings are consistent with a plesiomorphic and non-hearing organisation of sensory structures in T. neglectus, and do not directly support an evolutionary reduction of tympanal hearing organs.

OP-M-12

First report of co-occurring smooth and nubby euplantulae on a single tarsus: functional morphology approach to the attachment structures of Acanthoproctus diadematus (Orthoptera: Tettigoniidae: Hetrodinae)

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Insects have developed different structures to adhere to surfaces. Most common are smooth and hairy structures, while nubs have only been described for representatives of i. e. Mantophasmatodea, Phasmida and Plecoptera. Here we report on the structure and function of the attachment structures of the !Nara cricket Acanthoproctus diadematus. The three proximal euplantulae of each tarsus have a nubby surface, whereas the most distal euplantula is rather smooth with a hexagonal ground pattern that resembles that described for the Great Green Bush-Cricket Tettigonia viridissima. This is the first report of the occurrence of nubby euplantulae in Orthoptera as well as nubby and smooth euplantulae within a single tarsus. Recently it was shown that nubby structures generate relatively high friction forces, while smooth structures are mainly responsible for high adhesion (Bußhardt et al. 2012, Zoology; Labonte and Federle 2013, PLoS ONE). In these studies, contact forces on parts of a single euplantula were measured. We can experimentally confirm this functional difference in A. diadematus by the behavioural study, showing that when adhering to a wall, nubby euplantulae are in contact in a higher proportion compared to adhering to a ceiling. In contrast, adhering to a ceiling, smooth euplantulae are in contact in a higher proportion compared to adhering to a wall.
Neural basis of vocal communication in mammals - from behavior to physiology to cognition

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Vocal communication is a complex behavioral pattern that can be found in all mammalian species. It can be subdivided into learned vocal patterns such as human speech, and genetically pre-programmed vocalizations, which include those of non-human primates and most other mammals. One of the general aims of my research is to study and further understand vocal production and audio-vocal integration mechanisms in mammals as a whole, i.e., on each level of the vocal motor system. In my talk, I will give an overview on our recent studies on vocalization-related cognitive processes in the cerebral cortex, on innate brainstem mechanisms involved in audio-vocal integration processes and on intrinsic properties of the vocal organ, the larynx, itself. We performed these studies with a broad range of different methodological approaches including neurophysiological, neuroethological and psychophysical techniques on several animal models such as bats, squirrel monkeys and rhesus macaques. In the studies on vocalization-related cognitive processes in the cerebral cortex, we studied evolutionary aspects of human speech control with our newly established primate model and recorded single neurons in the vocalizing rhesus monkey. We found a neuronal correlate of volitional call initiation in the monkey homologue of human Broca’s area that suggests a cardinal role of this structure in vocal planning and call initiation, a putative phylogenetic precursor in non-human primates for speech control in linguistic humans. In contrast to the physiological perspective of question one, we study MET from the perspective of dysfunction with:

2) Which inner ear damages lead to the generation of tinnitus? We conduct experiments with Mongolian gerbils to identify critical factors and processes related to the development of tinnitus. Based on the observation that only part of our group of experimental animals develops tinnitus, we suspect one reason for the initiation of tinnitus in the signal transduction process and routing in the inner ear.

Auditory signal transduction and consequences of dysfunction

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Sound-induced waves lead to the opening of mechano-sensitive ion channels in the sensory cells of hearing organs. The resulting ion inflow changes the membrane potential of the neuronal cell. This process is called mechano-electrical transduction (MET). Although MET is a fundamental process in hearing and can be found in all parts of the animal kingdom, it is still not understood in detail. To gain insight, we address two major questions on signal transduction each related to a specific animal model. 1) What are the general principles of the MET in the ear? The hearing organ of bushcrickets, the *crista acustica*, is functionally similar to the basilar membrane of mammals. In contrast to the coiled cochlea, the straight shape of the *crista acustica* allows in-vivo experiments along the entire frequency range. To obtain information about the MET, we measure sound-induced motion of the hearing organ in addition to receptor cell responses. Using the *crista acustica* as a simple model system, we investigate the fundamentals of MET, including cellular micromechanics, tonotop interactions and mechanical reaction of sensory cells. These measurements provide important basic data for the study of hearing physiology in animals. In contrast to the physiological perspective of question one, we study MET from the perspective of dysfunction with: 2) Which inner ear damages lead to the generation of tinnitus? We conduct experiments with Mongolian gerbils to identify critical factors and processes related to the development of tinnitus. Based on the observation that only part of our group of experimental animals develops tinnitus, we suspect one reason for the initiation of tinnitus in the signal transduction process and routing in the inner ear.
OP-NB-01

The Diversity of Beetle Brains

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Worldwide approximately 1.4 million animal species have been described. Insects account for about 70% of the described species, with the coleopteran (beetles) making up one third of all animal species. This species richness of more than 400,000 beetle taxa is associated with an enormous diversity of adaptations reflected e.g. in the ecology, behavior and morphology of these animals. We assume, that this broad diversity is somehow reflected in the neuronal anatomy of the beetle brains.

To investigate the diversity of the beetle brains, we analyzed more than 60 beetle species, covering different aspects like phylogeny, feeding types, habitats, body sizes or movement types. To compare the neuroarchitecture of the various beetle brains, we performed immunostainings against synapsin and tachykinin-related peptides and used phalloidin and DAPI labelings.

Based on these stainings, we performed 3D-reconstructions of selected brain neuropils from more than 50 beetle species including the optic lobes, antennal lobes, central complex and mushroom bodies. Comparison of the relative volumes of the selected neuropils between the examined species revealed striking similarities within several families, while between families typically large differences occurred. In addition, we analyzed the relationship between body size and brain size and found convincing correlation between both.

OP-NB-02

Retrograde fills in Onychophora (velvet worms) shed light on the segmental identity and evolution of post-oral commissures in panarthropods

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As one of the closest arthropod and tardigrade relatives, Onychophora play an important role for understanding the evolution of panarthropods. To clarify the homology of the post-oral commissures between onychophorans and arthropods, we performed retrograde fills of the five anterior-most, post-oral commissures in the onychophoran Euperipatoides rowelli. Our data show that the neuronal cell bodies of the first post-oral commissure are mainly located in the deutocerebrum of the onychophoran brain as well as in the “connecting pieces” linking the brain with the nerve cords. At least some neuronal somata supplying this commissure are located in the contralateral brain hemisphere. Our additional fills of the post-oral commissures 2-5 also show anteriorly shifted somata, which is not the case in the remaining median commissures connecting the paired nerve cords. However, in contrast to the first post-oral commissure, none of the somata associated with the post-oral commissures 2-5 are located in the contralateral part of the onychophoran brain. This suggests a unique nature of the first post-oral commissure in Onychophora, although its role remains to be clarified. Interestingly, while our data indicate that the first post-oral commissure is associated with the second (deutocerebral) head segment in Onychophora, the first post-oral commissure of arthropods most likely belongs to the third (tritocerebral) segment. This implies that the first post-oral commissure is not homologous in onychophorans and arthropods and that the homolog of the first/deutocerebral commissure of onychophorans might have been either lost in arthropods, or incorporated into their brain.
**OP-NB-03**

**Some like it hot: isothiocyanate reception by *Phaedon cochleariae***

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Pungent odors like isothiocyanates (ITCs) which are emitted by plants belonging to the Brassicaceae taxon are used by some insects specialized on Brassicaceae as cues for host plant finding. Also the mustard leaf beetle *Phaedon cochleariae* is attracted by ITCs. We showed that adult *P. cochleariae* females preferred odors from ITC emitting plants only when they experienced these molecules during the pupal and early adult stage. To identify proteins involved in the reception of ITCs, we compared the antennal transcriptomes of beetles primed on a specific ITC and of an unprimed control group. Among those genes which were highly differentially expressed, we found an odorant binding protein (OBP1), a small molecule shuttling volatiles to the respective odorant receptor. Reverse genetic modification of OBP1 by RNAi resulted in a loss of preference for ITCs in beetles which were primed on this substance, indicating that expression of this OBP might be involved in priming of reception of ITCs. To prove if OBP1 indeed binds to ITCs, the protein was recombinantly expressed and used in a competitive binding assay using different ITC substrates. Yet, no ITC receptor has been identified in insects specialized on Brassicaceae. In *Drosophila melanogaster* ITCs are perceived by the receptor painless, a nociceptor belonging to the transient receptor potential (TRP) ion channel-family. This receptor is homologous to the mammalian “wasabi” receptor (TRPA1) whose involvement in ITC reception has been shown as well. By screening *P. cochleariae* antennal transcriptome data with sequences that we had obtained by a degenerated primer strategy, we were able to detect TRP sequences homologous to the known painless and TRPA1 sequences.

**OP-NB-04**

**Sensory physiology of two cold-sensitive neurons associated with the sensillum coelocapitulum in the ant *Camponotus rufipes***

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Social insects protect themselves from harsh environments by building elaborated nests that allow microclimatic control. Within the nest, ants care for their immobile brood by transllocating it to meet favorable thermal conditions. In behavioral tests, temperature differences of only 0.2°C can be discriminated, and brood is placed at temperatures close to 31°C. In addition, the thermal preference of an ant during brood-care is influenced by its own circadian rhythm, preferring higher temperatures in the afternoon (1, 2). The extremely fine-tuned brood-care behavior of ants illustrates how social insects can cope with the challenge to provide suited conditions for the brood.

Associated with the sensillum coelocapitulum (SCA), two thermo-sensitive neurons have been found in the carpenter ant *Camponotus rufipes*. One of these antennal cold-sensitive neurons is highly sensitive to temperature in a limited temperature range that matches the preferred brood temperature. We speculate that the neuron acts like a temperature switch (TS-neuron) and triggers the behavioral response during brood care. The second cold-sensitive neuron only responds to temperature changes with a phasic response and a slow adaptation (half-time:~30 sec; TT-neuron).

For both thermo-sensitive neurons, the dose response properties are currently analyzed with respect to circadian rhythm in order to test the hypothesis that the thermo-sensory system is modulated in such a way that it matches the behaviorally described circadian brood-care behavior.

OP-NB-05

One corazonin containing cell advances the circadian pacemaker of the Madeira cockroach *Rhyparobia maderae* possibly by relaying light information.

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The circadian pacemaker of the Madeira cockroach was located to the accessory medulla (AMe) and its approximately 250 associated neurons, which contain a striking number of different partly colocalized neuropeptides. Only one of these AMe neurons expresses corazonin, which functions in the circadian system are not known. The circadian clock is entrained to the daily light dark cycle via light entrainment pathways which are also not well characterized in the Madeira cockroach. Since injections of the neurotransmitter GABA, as well as injections of allatotropin, myoinhibitory peptides, and orcokinin resulted in light-like phase-response curves (PRCs), they were suggested to be part of the light entrainment pathway. Injection of corazonin in combination with running-wheel assays as well as double-label immunocytochemistry were performed to determine whether also corazonin could be involved in light entrainment of the circadian clock. *Rhyparobia*-corazonin generated an all-advance type PRC with significant advances between circadian times (CT) 18 and CT 21, reminiscent of light-dependent advances. Multiple-label immunocytochemistry demonstrated that the corazonin cell colocalized GABA and MIP, but not allatotropin. It belongs to the group of medial AMe neurons and terminated in a more proximal layer of the medulla, without direct contact to histaminergic fibers from the ipsilateral compound eye, but in close proximity to projections of light-sensitive neurons from the contralateral eye. We hypothesize that the corazonin cell relays light-dependent phase-advances to the clock during the late subjective night possibly integrating indirect ipsi- and contralateral light inputs. [Supported by DFG grant STE531/21-1 to MS]

OP-NB-06

Electrophysiological investigation of different methods of anesthesia in lobster

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Electrophysiological studies of neuronal processes and signal transmission in the CNS of lobsters during anesthesia are scarce. Therefore we tested four anesthesia methods and measured neuronal responses using implanted extracellular hook electrodes in the central nervous system, which were fixed with silicone. After our definition, full anesthesia was accomplished, if no neuronal response to different external stimuli was measurable in the CNS.

Electrodes were implanted into the CNS of young lobsters *Homarus gammarus* (20-30g) and adult lobsters *Homarus americanus* (450-800g). Four different anesthetization methods were investigated: (1) cooling in ice water (2) supplying CO₂ to the aquarium water (3) incubation in 10% MgCl₂ solution and (4) electrical stunning. Stimulation was performed using mechanical brushing, electrical stimulation, and heat treatment. Extracellular signals were recorded and analyzed in part by the Fast Fourier Transformation (FFT) to reveal the power spectrum and the frequency content of signals in the connectives. It is well known that cooling reduces metabolic rates. Visually we observed a reduced mobility of the animals (after 60 min at -1.8°C), but not an inhibition of the sensory response to stimuli. CO₂ needed a long time of exposure (15-60 min) and turned the animals immobilized. The neuronal transmission and sensory response was significantly reduced in most cases. MgCl₂-incubation for 1 h had no significant effect. Electrical stunning is promising but further work is necessary to reveal conclusive results.
OP-NB-07

Interaction between AKH and octopamine in the regulation of *Drosophila* locomotor behavior

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Vertebrate and invertebrate motor control is based upon neuronal activity in local circuits in the spinal cord or ventral nerve cord, respectively [1]. Descending neurons from the brain further regulate these neuronal circuits. In insects, the central complex as the major locomotor center was shown to control different aspects of locomotor activity such as velocity, activity maintenance and orientation [2]. In addition to the central complex, the mushroom bodies are suggested to participate in locomotor control as they were shown to trigger walking behavior [3]. Neuropeptides, key players in the adaptation of neuronal networks to environmental changes, can modify and orchestrate complex behaviors and were shown to influence the modulation of locomotor behavior. In detail, adipokinetic hormone (AKH) is known to function in the regulation of hemolymph lipid and carbohydrate levels, thus required mainly under starvation or acute locomotor activity for energy mobilization [4]. In this study we focus on the function of AKH for locomotor activity of flies and figure out potential aminergic targets of this peptide hormone.


OP-NB-08

Coping with the stream - bulk flow sensing in rheophilic fish

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Fish sense weak water motions induced e.g. by conspecifics, prey, predators, objects located upstream or bulk water flow with the mechanosensory lateral line. Here, we studied the flow sensing capabilities of the rheophilicide (*Leuciscus idus*) using electrophysiological recordings from primary lateral line afferents and brainstem neurons, investigations of peripheral lateral line morphology and hydrodynamic simulations. We presented near-laminar flow (1.8 to 9.5 cm/s) avoiding hydrodynamic disturbances caused by artificial ventilation or surface waves. We found neurons that increased their spike rate in bulk water flow, whereas other neurons did not. Stronger increases in spike rate were observed at higher bulk flow velocities. When bulk flow direction was reversed, some units showed a pronounced increase of spike rate to one but not (or at least much weaker) to the other flow direction. Thus, these units were flow direction sensitive. This directionality was most obvious at flow velocities up to about 4 cm/s and occurred preferentially in neurons with a receptive field near the head of the fish. Morphological analysis of the cranial lateral line in ide indicated that at least some sections of the anterior canal system are involved in bulk flow sensing. E.g., the cranial lateral line canals were directed towards the front of the head and the diameter of the frontal-most canal pores was notably smaller than the canal lumen. We transferred the basic morphological features into a simple model and simulated the hydrodynamic effects of bulk flow on the cranial canals. The results imply that the anterior canal system provides valuable information for determining bulk flow velocity with high resolution.
OP-NB-09

Adaptive processes in the fish lateral line system

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Adaptation is a fundamental property of all sensory systems. It is characterized by a decrease in responsiveness to a single constant-amplitude stimulus or to a sequence of repetitive stimuli. Adaptation has been well-studied in nearly all hair-cell based systems of many vertebrates, but has thus far not been systematically investigated in the lateral line system.

We study neuronal adaptation to single stimuli and to repetitive stimulation in both, the peripheral and central lateral line. To do so, we record neuronal responses of primary afferent nerve fibers and units in the lateral line brainstem to hydrodynamic dipole stimuli generated by a sinusoidally vibrating sphere. In this presentation, we summarize our present findings.

We found that both, primary afferents and brainstem units can exhibit short-term adaptation to single constant-amplitude stimuli. The degree of adaptation depended on stimulus frequency and, for afferent fibers, also on stimulus amplitude. In addition, peripheral and central lateral line units can show adaptation to a series of successively presented stimuli, i.e. the evoked discharge decreased systematically with increasing stimulus number. Moreover, both the numbers of units that exhibited a rate decrement and the percentage of rate decrement increased with decreasing time intervals between successive stimuli.

Our data are in agreement with previous studies on neuronal adaptation in peripheral and central auditory neurons in other vertebrates. In future studies we want to investigate the relative contribution of different stages of the ascending pathway to adaptive processes in the lateral line system.

OP-NB-10

Expression Plasticity of Opsin Genes in *Camponotus rufipes* workers

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*Camponotus rufipes* workers start to forage outside the nest after a period of about 4 to 6 weeks inside the colony. Outside the nest, vision plays presumably a more important role for foragers that need to navigate in a light environment. In the present study we investigate if the transition from indoor to outdoor activity is accompanied by changes in opsin expression and therewith spectral sensitivity. Using qRT-PCR method, we aimed to answer: Which opsin genes do *C. rufipes* workers express? What is the expression plasticity of opsin genes in relation to age and light?

Our results showed that *C. rufipes* workers expressed long-wavelength (LW1), ultraviolet (UV) and blue (BL) opsin mRNAs at varying levels, respectively, from most to least expressed levels. Phylogenetic analyses revealed that the inferred amino acid sequences were similar to visual opsin sequences of other insects such as *A. mellifera* and *C. floridanus*. Considering the plasticity in opsin gene expression in relation to age, Post hoc analysis showed that expression levels of all three opsins increased up to 14 days of age and after this age showed no significant increase.

As a conclusion, *C. rufipes* workers, like many other hymenopterans, express three opsins (LW1, UV, and BL) in their compound eye and show expression plasticity in relation to age and extended light (except BL). Opsin expression is regulated by multiple factors at the same time such as light, age and circadian clock, as shown in honey bees, and therefore expression plasticity of opsins is strongly related to environmental factors and physiological adaptations.

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OP-NB-11

Unraveling the cellular properties of neurons involved in coordination of central pattern generators

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We use the crayfish swimmeret system as a model to study coordination of neural oscillators because exactly three identified neurons in each hemiganglion are necessary and sufficient to coordinate the posterior to anterior metachronal wave of swimmeret movement: One ascending (ASCₑ) and one descending (DSC) coordinating neuron as well as one Commissural Interneuron 1 (ComInt 1). The coordinating neurons ASCₑ and DSC encode information about the activity state of their home segment and send it to the ComInt 1s of the other segments, where the information is decoded and integrated into the swimmeret’s central pattern generator (CPG). This CPG also shapes ASCₑ’s and DSC’s activity. Now that the coordinating circuit is identified on the neuronal level we investigated specific cellular properties that allow the coordinating neurons to encode information at different excitation levels. We recorded ASCₑ intracellularly in an isolated nervous system preparation (chain of six abdominal ganglia) and perfused it with different concentrations of carbachol, a cholinergic agonist, to set the excitation levels. Noteworthy is that the phase and membrane potential of ASCₑ’s first action potential (AP) was stable across excitation levels. On the other hand, the phase and membrane potential of the last AP was variable. Therefore, we propose that the onset of activity is highly fixed by the network properties. We also proved that carbachol acted both directly and indirectly on ASCₑ’s number of open ion channels. This opposing effect may contribute to a mechanism that stops ASCₑ’s firing, allowing the precise encoding of coordinating information.

OP-NB-12

MOTION VISION IN HARBOR SEALS (PHOCA VITULINA)

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Motion perception is one of the fundamental properties of the visual system, and it is most likely of high importance for e. g. object detection and avoidance even in semi-aquatic mammals such as in harbor seals. In a series of experiments we have investigated underwater motion vision in harbor seals, the first approaches to motion vision in a marine mammal. In experiment 1, the optokinetic nystagmus as a basic motion sensing and retinal image stabilizing reflex was studied. All harbor seals showed optokinetic eye movements when presented with a moving black-and-white stripe pattern, and the seal eye stabilized visual motion equally well irrespective of movement direction. In experiment 2, the seal’s sensitivity to global motion was analyzed using random dot displays. Coherence thresholds between 5 - 30% were obtained with the lowest thresholds comparing to the performances of monkeys and humans. With this high sensitivity to global visual motion, harbor seals could benefit e. g. from optic flow information. Optic flow perception was thus investigated in experiment 3 in which a forward movement through a cloud of dots was simulated on a large projection to a seal. The seal was able to perceive optic flow and could detect deviations from the simulated heading with high accuracy. All these studies imply that the seal eye is very sensitive to visual motion and that the interpretation of global motion might play an important role for locomotion and orientation in their environment even in turbid waters where vision has been considered to be limited until now.
OP-P-IT-01

Making light work of body weight regulation: Photoperiod, retinoic acid signalling and inflammation

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Seasonal animals use photoperiod as a cue to time long-term and reversible changes in physiology such as food intake, growth and energy balance. Recent work indicates that these changes involve altered thyroid hormone and retinoic acid signalling within the ependymal cells of the hypothalamus. In this study we examined signalling downstream of retinoic acid signalling, and investigated how it links to the control of energy metabolism and growth. Using microarray analysis and quantitative PCR array analysis, we identified gene expression changes in the hypothalamus of photoperiod-sensitive F344 rats in response to photoperiod and retinoic acid. The most significant changes were those related to inflammatory pathways, and we identified a wide number of chemokines and cytokines, such as the recently identified chemokine chemerin, known to be involved in the regulation of energy metabolism and inflammation. Intracerebroventricular injections of chemerin altered body weight and food intake, accompanied by a change in peptidergic modulators that play a pivotal role in growth and feeding. Furthermore we found that the number of CD11b stained microglia, indicating activated brain macrophages, in the hypothalamus were higher in long as compared to short day photoperiod suggesting that the changes in inflammatory signalling may be a part of the innate immune response. Together these results support the novel finding that inflammatory signalling is strongly up-regulated in the hypothalamus of seasonal rats in response to photoperiod and retinoic acid signalling and suggest that inflammation is likely to play a major role in seasonal changes in growth and energy balance.

OP-P-IT-02

When the going gets hot & sour - can mitochondria set the cellular basis for acclimation in a changing ocean?

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Ocean acidification goes hand in hand with ocean warming and increasingly threatens life in the world’s oceans. Due to their well-developed capacities for ion- and pH-regulation, fish have generally been regarded as reasonably tolerant towards ocean acidification. Yet, there is a growing body of evidence that fish - and especially so their early life stages - also have to rearrange their aerobic metabolic pathways to meet the challenges of ocean acidification and warming, including energetic trade-offs. This puts their mitochondria into the focus of interest, which have to provide aerobic energy in sour times of increased temperatures and PCO₂. I will present examples of how these challenges are met in mitochondrial acclimation of various life stages of cold temperate fish species. Maternal effects also become visible at the mitochondrial level and can additionally serve to use the full range of genotypic and phenotypic plasticity in physiological acclimation to a changing ocean.
OP-P-01

Structure and function of pinniped body surfaces

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The semi-aquatic lifestyle poses a great challenge to the group of pinnipeds, since they have to maintain a constant body temperature in water and air despite the highly differing thermal properties of these two media. Therefore pinnipeds have evolved different types of thermal insulation consisting in fat (blubber) and/or fur, forming a variety of different surface structures. Surface structures differ not only between species, but also between body parts and life phases, and they most likely have additional functions beside thermal insulation. We investigated body surfaces in two different pinniped species, the harbor seal (Phoca vitulina) and the Cape fur seal (Arctocephalus pusillus pusillus), with regard to their structure, properties and possible functions.

Newborn fur seals are covered with a special natal coat that differs significantly from the adults’ fur. This fur protects the pups during their first terrestrial life phase, exposed to high temperatures and intense solar radiation. Harbor seals contrarily are mainly insulated with blubber and possess a sparse fur that forms a smooth body surface. The fur’s thermal insulating properties have been regarded as negligible so far. We found that this does not apply to all body parts, since the seals’ vibrissal pads are covered with fur of a unique structure that has not been described in any mammal to date. Furthermore, the skin of harbor seals has a unique structure as well, consisting of scale-like flexible lobes. Both fur and skin structure presumably serve not only thermal insulation but also drag reduction.

OP-P-02

Anaerobic capacity of the brain of an expert diver, the hooded seal (Cystophora cristata)

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Due to its high metabolic activity, the mammalian brain is very sensitive to hypoxia. The brain’s energy metabolism mainly relies on aerobic energy production by oxidative phosphorylation. However, during the dives the brains of many marine mammals withstand the hypoxia-induced energy challenge without deleterious effects. The cerebral hypoxia tolerance of diving mammals was thought to be at least partially achieved by an enhanced capacity of anaerobic energy production. While some studies have reported a high anaerobic capacity in the brains of diving mammals, the mechanisms of adaptations are still unknown. Lactate dehydrogenase (LDH) is the key enzyme of the anaerobic metabolism and is composed of two distinct subunit types (LDH A and B), which form five distinct tetramers with different subunit compositions and properties (LDH-1 to -5). We have compared the sequences, mRNA and protein expression levels, enzyme activity and isozyme distribution of LDH in the brains of the hooded seal (C. cristata), the ferret (Mustela putorius furo) and the mouse (Mus musculus). The highest LDH mRNA levels were found in the ferret brain. The highest LDH activity was observed in the hooded seal brain. The isozymes distribution corroborates the findings of the activity assay. The highest LDH A content was found in the ferret brain. The LDH B content did not differ among the brains of these species. Since the LDH A and B sequences of the hooded seal showed no alterations that could explain a higher enzyme activity, we speculate that the difference in enzyme activity is due to post-translational regulations.

Keywords: hooded seal’s brain, anaerobic capacity, lactate dehydrogenase

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OP-P-03

**Proton-driven Metabolite Transport is facilitated by Carbonic Anhydrases**

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The proton-linked monocarboxylate transporters MCT1-4 play a crucial role in the shuttling of lactate between glycolytic and oxidative cell types in different tissues like muscle and brain. By measuring changes in intracellular H⁺ concentration with H⁺-sensitive microelectrodes in MCT-expressing *Xenopus* oocytes, we demonstrated that carbonic anhydrase isoform II (CAII) enhances transport activity of MCT1 and MCT4, while leaving transport activity of MCT2 unaffected. CAII-induced augmentation of MCT1/4 transport activity is independent of CAII catalytic activity, but is mediated by the enzyme’s intramolecular H⁺-shuttle His64 (Becker et al., 2008, 2011). H⁺ transfer between enzyme and transporter requires close proximity of the two molecules. This is achieved by direct binding of CAII to the transporter’s C-terminal tail via the acidic amino acid clusters E489EE in MCT1 and E439EE in MCT4. Coexpression of MCT1, MCT2 and MCT4 with extracellular located CAIV also results in a significant increase in lactate transport by MCT, independent of CAIV catalytic activity. The effects of cytosolic CAII and extracellular CAIV on MCT transport activity were additive. Our results suggest that intra- and extracellular carbonic anhydrases can act as proton-collecting antennae for the MCTs. These antennae dissipate local H⁺-microdomains at the transporter pore to ensure enhanced transport activity and rapid shuttling of metabolites across the cell membrane.

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References


OP-P-04

**Dietary composition of unsaturated fatty acids is of minor importance for the occurrence of daily torpor in the seasonal dwarf hamster *Phodopus sungorus***

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Essential polyunsaturated fatty acids (PUFA) have been shown to play a particular role in hibernating mammals. In contrast, the relevance of dietary PUFA for daily heterotherms exhibiting only brief and shallow torpor bouts is less well studied. Therefore, special diets differing in PUFA composition were used to examine the effects on the incidence of spontaneous daily torpor in Djungarian hamsters (*Phodopus sungorus*). Although we found a slight positive effect on the torpor frequency in hamsters fed a diet rich in n-6 PUFA compared to the groups fed diets either rich in n-3 PUFA or monounsaturated fatty acids, the latter did not show unusually low torpor frequencies. The results of the additional diet choice experiment indicated that hamsters in short photoperiod select food with only a slight excess of n-6 PUFA compared to n-3 PUFA (ratio of about 1 to 1.5). However, there was no significant difference in torpor frequency between the diet choice group and hamsters fed the standard chow with a sevenfold excess of n-6 PUFA. Based on the precondition that there is no nutritional deficiency in essential fatty acids, the present data strongly indicate that the dietary composition of unsaturated fatty acids plays a minor role for the occurrence of spontaneous daily torpor in Djungarian hamsters.
OP-P-05

Maintaining a homeostatic balance in the fruit fly’s intestine

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The intestine of Drosophila is a single layered epithelium composed of only few different cell types that shelters an endogenous microbial consortium of low diversity. This microbiome is of particular importance for digestion of sugars. Maintaining the homeostatic interaction between microbiome and epithelium is of prime importance to keep a healthy epithelium and the balance between cell apoptosis and cell renewal in the gut. Controlling the activity of intestinal stem cells (ISCs) is of prime importance to replenish the pools of enterocytes and that of hormone producing enteroendocrine cells over time. This homeostatic situation can be challenged by different interventions including infection with bacterial pathogens or by feeding a specific high fat diet. We could show that these interventions lead to changes in different parameters including overall activity and longevity. Moreover, high fat dieting has a long lasting effect on the microbial community leading to a massive increase in intestinal microbes. In addition, these nutritional interventions led to a cytokine-induced activation of stem cell activity that modified the gut structure chronically. On the other hand, major physiological parameters of the intestine are controlled by endogenously produced hormones. The major aim of this project was to understand this complex interplay between microbiota, the intestinal physiology and the different nutritional challenges, leading to a homeostatic balance.

OP-P-06

Synthesis of salivary proteins upon feeding in salivary gland cells of the medicinal leech, Hirudo verbana

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Saliva of hematophagous parasites contains bioactive substances, which allow the animal proper feeding, but may also exert beneficial effects in the host. An interesting annelid in this context is the medicinal leech, Hirudo verbana, which is used for medical purposes since ancient times. Its saliva contains of a complex composition of salivary proteins and peptides that allows the animal to inhibit blood coagulation and to prevent pain sensations in the host during feeding and to store ingested blood in its crop for extended periods of time. Upon feeding, the storage compartments of the unicellular salivary gland cells are virtually empty (Hildebrandt & Lemke (2011) Naturwissenschaften 98, 995-1008). To prepare for an additional blood meal, the leech has to refill these cell compartments by synthesis of salivary proteins and peptides. We set out to investigate the time course of salivary protein synthesis and refilling of the salivary gland cells. We fed animals with citrate blood drawn from pigs and fixed the anterior body part of leeches including the salivary gland tissue at one, three and five days or one, two or three weeks, respectively, upon feeding. We used Azan-stained longitudinal sections (5 μm) of leech tissue to determine the number and size (mean area) of salivary gland cells. The results indicate that synthesis of salivary proteins and peptides sets in early upon feeding (within 1 week). At 3 weeks upon feeding, the salivary gland cells appear as those in unfed control leeches.
OP-P-07

Juvenile hormones in the control of semelparous reproduction.

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Many strategies have evolved to ensure successful reproduction. One of the most fascinating ones is the occurrence of semelparity, an all-or-nothing strategy of reproduction finally leading to the death of the individual animal. Semelparous reproduction often culminates in mass spawnings as they are observed in many aquatic species such as corals or salmon. Concomitant with this is a massive generation germ cells, representing up to 80% of the individuals` body mass at spawning. To secure a maximum of reproductive success, the presence of mature gametes has to be synchronised in the individual as well as in the population.

In the polychaete Platynereis dumerilii, sexual maturation - and thereby synchronised gametogenesis - has been linked to the sinking titer of a yet uncharacterised hormone termed nereidin that has been paralleled with insect juvenile hormones. Nereidin has a central role in triggering sexual maturation and the concomitant metamorphosis of the animal from a feeding and growing animal to a non-feeding, reproducing one. However, nothing is hitherto known about its molecular nature and mode of action. Eleocytes (free coelomic cells), represent the central metabolic organ in Platynereis eventually taking over the main metabolic load associated with maturation and are essential for this process. As a massive redistribution of stored energy from the soma to the germ line accompanies sexual maturation, we have chosen to focus on the eleocyte to elucidate the molecular nature of nereidin and its impact on vitellogenesis.

OP-P-08

The pygmy grasshopper, *Tetrix subulata*, synthesises a novel adipokinetic octapeptide with a third aromatic amino acid in its corpus cardiacum

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Adipokinetic hormones (AKHs) are produced in neurosecretory cells of the corpus cardiacum (CC) of insects. They are multifunctional but the most important regulatory role is the control of energy metabolism during periods of locomotory activity. They are octa- to decapeptides. About 60 different primary structures of AKHs have been identified. In caeliferan Orthoptera the AKHs of three of the advanced superfamilies (Pyrgomorphoidea, Pneumoroidea, Acridoidea) are known but there is still little information available on the basal superfamilies (Gäde and Marco, Gen Comp Endocrinol 162, 59-68, 2009). Here we report on the complement of AKHs in the Tetrigoidea. Isolation and sequencing of two AKHs from the CC of the pygmy grasshopper, *Tetrix subulata*, was achieved by liquid chromatography-electrospray ionisation mass spectrometry (LC-ESIMS) and/or RP-HPLC with Trp-fluorescence monitoring: (1) pGlu-Leu-Asn-Phe-Ser-Thr-Gly-Trp amide, code-named Schgr-AKH-II, formerly found in *Schistocerca gregaria* and (2) pGlu-Phe-Asn-Phe-Thr-Pro-Gly-Trp amide, a novel sequence now code-named Tetsu-AKH. The structure of Tetsu-AKH is unusual; only two other AKHs with a Phe at position 2 were previously found in certain dung beetles (code-named Scade-CC-I and II) and they differ from Tetsu-AKH at three positions, viz. residues 4, 5 and 7. There is an AKH, Psein-AKH, with a conservative single point mutation from Phe² in Tetsu-AKH to Val². It occurs in zygopteran Odonata species and in some families of Mantophasmatodea which belong as the Orthoptera to the Polyneoptera. It is not known how Tetsu-AKH and Schgr-AKH-II evolved in *T. subulata* by gene duplication and mutation since three changes at positions 2, 3 and 6 are required.

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**OP-P-09**

**Daytime-dependent modulation of pheromone detection in the hawkmoth *Manduca sexta***

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The crepuscular hawkmoth *Manduca sexta* is active at night while it rests during the day. In synchrony with their rest-activity cycle females release maximum amounts of a sex pheromone blend during night to attract their conspecific mates. Males detect the pheromones with long, antennal trichoid sensilla which are innervated by two olfactory receptor neurons (ORNs) each. Always one of the two is tuned to bombykal (BAL) the main sex pheromone component. Corresponding to behavioral rhythms BAL-sensitivity of male ORNs varies daytime-dependently being highest during the activity phase. With extracellular tip recordings and ELISAs we examined molecular mechanisms of daytime-dependent changes in antennal pheromone sensitivity. Previously, it was shown that the stress hormone octopamine (OA) sensitizes BAL responses daytime-dependently. Thus, we examined whether OA concentrations cycle in the antenna as possible basis for rhythms in olfactory sensitivity. With ELISAs we showed that OA increases concentrations of IP₃ and cAMP in the hawkmoth antenna. Furthermore, daytime-dependent changes in OA-, IP₃-, and cAMP levels occurred. In contrast to the synchronized peaks of OA and cAMP, IP₃ peaks rather correlated with maxima in olfactory activity. Accordingly, BAL-stimulation causes daytime- and protein kinase C (PKC)-dependent rises in IP₃ levels. Furthermore, PKC agonists enhanced BAL-sensitivity in tip recordings daytime-dependently. Therefore, we suggest that next to oscillations in OA-levels the state of phosphorylation determines the sensitivity of the olfactory system in *M. sexta*. [Supported via DFG SPP 1392, STE 531/20-1,2 to MS]

**OP-P-10**

**A tiny beetle and it´s big asset - The Senecionine Monoxygenase of *Longitarsus jacobaeae***

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Although the flea beetle *Longitarsus jacobaeae* is a tiny and very unimpressive insect, it is highly interesting for being one of very few animals that are specialized on tansy ragwort (*Senecio jacobaeae*). This common wild flower is known to be highly toxic, not only for insects but also for cattle and horses due to its content in pyrrolizidine alkaloids, mainly senecionine-N-oxide. This compound serves (the plant) as defense mechanism against herbivores and is responsible for hepatotoxic or even lethal effects for farm animals.

In this study we investigated which physiological adaptions allow *Longitarsus jacobaeae* to feed unharmed on *Senecio jacobaeae*. The challenge in dealing with pyrrolizidine alkaloids consists in their conversion from N-oxides into tertiary alkaloids in the herbivores’ gut which then bring about the toxic effects. We identified sequences in the beetle’s transcriptome that are similar to known flavin-monooxygenases. Based on this information we were able to express and harvest the recombinant protein in Sf9 cells. Activity tests with tertiary senecione and the co-factor NADPH +H⁺ demonstrated that the flea beetle has a senecionine-monoxygenase able to specifically convert tertiary senecionine into the harmless N-oxide and furthermore that this enzyme is more active than the previously described enzyme of the arctiid moth *Tyria jacobaeae*. The main tissue for this metabolism could be identified by quantitative real-time PCR.
OP-P-11

Antibacterial activity of two crustacean lipoproteins, the large discoidal lipoprotein and the high density lipoprotein/beta-glucan binding protein.

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The large discoidal lipoprotein (dLp) of the crayfish, Astacus leptodactylus, differs from other crustacean hemolymph lipoproteins in its large size, apoprotein composition and high lipid binding capacity. We have recently identified an N-terminal lipid binding domain and a DUF 1943 domain in the dLp sequence suggesting a relationship with the large lipid transfer proteins. Since some of the members of this family display antibacterial activities, we have tested the dLp and another hemolymph lipoprotein, the high density lipoprotein/beta-glucan binding protein (HD-BGBP) in a bacterial growth inhibition assay. E. coli and Micrococcus luteus as representatives of a gram negative and gram positive species, respectively, were exposed to various lipoprotein concentrations (0-200 µg/ml medium) and incubated for 20h at 37ºC. Bacterial growth was monitored at 590 nm.

DLp inhibited E. coli significantly at 12.5 µg dLp/ml (25% inhibition) and completely at 200 µg/ml; HD-BGBP inhibited E. coli significantly at 25 µg dLp/ml (25% inhibition) and by 90% at 200 µg/ml. The growth of M. luteus was significantly inhibited by dLp at 100 and 200 µg/ml (75% inhibition); HD-BGBP inhibited the growth of this species by 30% at 50 µg/ml and complete inhibition was found at 100 and 200 µg/ml. Thus, both lipoproteins exhibited antibacterial activity with a stronger action of the dLp and the HD-BGBP against the gram negative and gram positive species, respectively.

Our recent sequencing of two additional dLp related proteins in A. astacus and Macrobrachium rosenbergi as well as the finding of dLp like EST-sequences in other crustaceans suggests a common antibacterial function of the dLp like proteins in the innate immune system of at least the decapod crustaceans.

OP-P-12

Rapid incorporation of glucosinolates as strategy used by a herbivore to prevent activation by myrosinases

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Several plants have a binary defence system consisting of a substrate and an enzyme, which only come into contact after tissue disruption, thereby forming reactive hydrolysis products. Insects feeding on such plants have to overcome this binary defence system or must prevent the activation. For insects feeding on Brassicaceae, they have to avoid the activation of myrosinases, which hydrolyse the characteristic metabolites of this family, glucosinolates, into toxic breakdown products. We studied how larvae of the sawfly Athalia rosae (Hymenoptera: Tenthredinidae) are able to deal with the binary defence system of their hosts, investigating myrosinase activities as well as glucosinolate concentrations in different sections of the insect body and gut. In the front part of the larval gut, only low myrosinase activities were found, whereas glucosinolate concentrations were high. Throughout the gut passage, myrosinase activities increased but glucosinolate concentrations became reduced. Using bioassays as well as Matrix Assisted Laser Desorption Ionization Mass Spectrometry Imaging (MALDI-MSI), we could demonstrate that the glucosinolate sinainbin is rapidly incorporated into the haemolymph within less than five minutes of feeding. Furthermore, MALDI-MSI revealed that the glucosinolate accumulates around the Malpighian tubules. The study highlights the pivotal role of the gut of an adapted herbivore as a regulatory functional organ to cope with the binary defence of Brassicaceae. MALDI-MSI may be also applied to other insect systems to study the fate of plant metabolites in the insect bodies.
OP-ZS-IT-01

The Orthoptera Tree of Life: Elucidating patterns and processes of orthopteran evolution

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Orthoptera is the most diverse order among the lower neopteran groups and includes familiar insects, such as grasshoppers, crickets, katydids and their kin. Due to a long history of conflicting classification schemes based on different interpretations of morphological characters, the phylogenetic relationships within Orthoptera are poorly understood and its higher classification has remained unstable. In this study, we establish a robust phylogeny of Orthoptera based on 36 of 40 families representing all 15 superfamilies and complete mitochondrial genomes and four nuclear loci to test previous phylogenetic hypotheses and to provide a framework for the natural classification and a reference for studying the pattern of divergence and diversification. We find strong support for monophyletic suborders (Ensifera and Caelifera) as well as major superfamilies. Our results corroborate with most of the higher-level relationships previously proposed for Caelifera, but suggest some novel relationships for Ensifera. Using fossil calibrations, we provide divergence time estimates for major orthopteran lineages and show that the current diversity has been shaped by dynamic shifts of diversification rates at different geological times across different lineages. We also show that mitochondrial tRNA gene orders have been relatively stable throughout the evolutionary history of Orthoptera, but a major tRNA gene rearrangement occurred in the common ancestor of Tettigioidea and Acridomorpha, thereby representing a robust molecular synapomorphy, which has persisted for 250 million years.

OP-ZS-IT-02

Comparative insect transcriptomics and its value for systematics

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The basis of insect diversity is a major question in modern biology. In the past, comparative transcriptomic studies were limited to model organisms, but the advent of next generation sequencing (NGS), combined with continuous progress in RNA-seq data quantity and quality, enables comprehensive studies of the entire transcriptome regardless of the availability of reference genome information. However, transcriptomic data - especially across developmental stages are mainly available for derived holometabolous insects. Therefore, we are currently filling in an important gap for future comparative transcriptomic studies by generating and analyzing data across the whole embryonic development of the damselfly Ischnura elegans. Using the Smart Seq 2 protocol for ultra-low RNA input and Tn5 based library synthesis we sequenced the transcriptonal profile of single embryos for each of the nine embryonic days on the Illumina HiSeq platform. Pilot analyses reveal three switches of transcriptional activity, identified by the transcriptome relationships among the 9 stage-specific profiles during Ischnura development. The new development-dependent transcriptomic information of this non-model organism will not only provide resources to quantify variability in gene expression across developmental stages within a species, it will also help to further study in detail an essential large scale developmental paradigm in developmental biology - the molecular signatures of embryonic developmental constraints and the parallels between phylogeny and ontogeny.
OP-ZS-01
A fresh look at ancient cells: mandibulate ground pattern and crustacean-hexapod-relationships based on serotonergic neurons.
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A close phylogenetic relationship of Crustacea and Hexapoda is now widely accepted, however the identity of the crustacean sister group to Hexapoda still remains elusive. Numerous, often contradicting, hypotheses have been postulated based on different kinds of analyses, such as morphological comparisons and molecular sequencing information. Another approach comparing neuroanatomy and development has regained attention in the last decade, with the intention to generate independent data for phylogenetic analyses. In this context, the distribution and projection pattern of serotonin-immunoreactive neurons in the ventral nerve cord is well suited for phylogenetic comparisons because of low numbers of these individual identifiable neurons and large amounts of available data for diverse arthropod taxa. However, data on basal hexapods are scarce. Here, we present the distribution and morphology of serotonergic neurons in the ventral nerve cord of the basal hexapod taxa Zygentoma and Archaeognatha based on immunocytochemical labeling of serotonin and tryptophan hydroxylase. Additionally, we performed preincubation experiments with neurotransmitter-related compounds in order to describe the distribution of the serotonin transporter and biosynthetic enzymes. A comparison of the ground patterns of immunocytochemical stainings to existing data is consistent with a close relationship of Remipedia to Hexapoda. A re-investigation of serotonin-immunoreactive neurons in the VNC of Chilopoda reveals that several aspects of the anatomy of serotonergic neurons traditionally assigned to the ground pattern of Tetraconata are already present in the ground pattern of Mandibulata.

OP-ZS-02
New mandible articulations in the earliest hexapod lineages
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The morphology of the mandibles and their articulation with the head capsule is a frequently used character system in hexapod systematics. It is currently assumed that the primary wingless insect lineages (Protura, Collembola, Diplura, Archaeognatha and Zygentoma) exhibit two mandible types. Monocondylic mandibles are principally composed of one posterior articulation, dicondylic mandibles show an anterior and posterior articulation. It has already been shown that this view is only partly true, several studies reported additional mandible articulations in Collembola and Archaeognatha or clarified the structural composition of already known articulations.

Here we report additional mandibular articulations in Collembola, Diplura and Archaeognatha. Collembola and Diplura each show an articulation of the maxillar stipes with the mandible. Archaeognatha possess two anterior mandible articulations, one with the head capsule and another one with the first maxillar palpus segment. Although these discoveries are difficult to interpret from a phylogenetic point of view, the character system mandible articulation is obviously more variable than previously assumed.
OP-ZS-03

The morphology of the ensiferan thorax and its phylogenetic implications

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The phylogenetic relationships among the species-rich ensiferans (crickets and katydids) have remained largely unexplored in the past, although some evolutionary aspects, such as the single or multiple origin of bioacoustic communication in these insects, are discussed controversially and with great interest among evolutionary biologists. Back in 1939, Kjell Ander already stated that skeletal and muscular features of the ensiferan thorax are highly informative in understanding phylogenetic relationships of this diverse taxon. Nevertheless, only fragmentary knowledge exists in this regard, which is mainly based on exemplary anatomical studies of few model organisms, e.g. the house cricket Acheta domesticus and the European mole cricket Gryllotalpa gryllotalpa. Here we present preliminary data about the thorax anatomy of previously unstudied ensiferans, demonstrating that this character complex can contribute significantly to the understanding of their phylogeny, e.g.: (1) In all investigated ensiferan species, the prothoracic muscle Ipcm8 (Musculus propleura-trochanteralis), a muscle running from the prothoracic pleura to the trochanter, consists of two clearly defined muscle bundles: one originates from the episternal face of the pleura, the other arises from the epimeral face of the pleura. (2) A sistergroup relationship of crickets (Gryllidae) and mole crickets (Gryllotalpidae) is supported by the specific course of the anterior bundle of the muscle Idvm19 (Musculus pronoto-trochanteralis). Interestingly, this bundle runs between the saddle-shaped pronotum and the internal, dorsally prolonged propleura (cryptopleura), therefore lying outside the pleural skeletal elements. (3) Cave crickets (Rhaphidophoridae) are characterized through an additional, longitudinally orientated median sclerite situated between the meso- and metasternum.

OP-ZS-04

Phylogenomic analyses of whole genome shotgun data unravels the origin and spread of pandemic Wolbachia bacteria in arthropods

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Wolbachia is probably the most common obligate intracellular bacterium. In general, most Wolbachia strains are either pandemic, opportunistic reproductive parasites of arthropods or essential mutualists in a single group of filarial nematodes, including many species of medical significance. Besides this, some strains are limited to a restricted set of taxa, often with an unknown lifestyle. A robust phylogenetic backbone of Wolbachia is lacking to date and consequently, many Wolbachia-related phenomena cannot be discussed in a broader evolutionary context. We here present the first comprehensive phylogenomic analysis of Wolbachia supergroup relationships based on new whole-genome-shotgun data. Phylogenomic analyses are conducted on a carefully selected set of orthologous genes and a whole set of sensitivity analyses (e.g., to test for LBA artifacts) have been carried out. Our results suggest that Wolbachia has evolved from a specialized, probably mutualistic ancestor and that major host switches have occurred from arthropods to nematodes and back. The ability of some arthropod infecting Wolbachia to adopt to new hosts quickly has likely evolved only once and enabled their pandemic spread. In the light of these results, hypotheses on the evolution of mutualism in Wolbachia need to be re-evaluated.
OP-ZS-05

Analysis of the opsin repertoire in the tardigrade *Hypsibius dujardini*: insights into the evolution of opsin genes in Panarthropoda and Bilateria

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Screening of a deeply sequenced transcriptome of the tardigrade *Hypsibius dujardini* revealed a set of five opsin genes. To clarify the phylogenetic position of these genes and to elucidate the evolutionary history of opsin genes using maximum likelihood and Bayesian inference methods in conjunction with carefully selected substitution models. According to our findings, the opsin repertoire of *H. dujardini* comprises representatives of all three major bilaterian opsin clades, including one r-opsin, three c-opsins, and a Group 4 opsin (neuropsin/opsin-5). The identification of the tardigrade ortholog of neuropsin/opsin-5 is the first record of this opsin type in a protostome, but our screening of available metazoan genomes revealed that it is also present in other protostomes. Our opsin phylogeny further suggests that two r-opsins, including an "arthropsin", were present in the last common ancestor of Panarthropoda. While both r-opsin lineages were retained in Onychophora and Arthropoda, the "arthropsin" was lost in Tardigrada. The single (most likely visual) r-opsin found in *H. dujardini* supports the hypothesis of monochromatic vision in the panarthropod ancestor, whereas two duplications of the ancestral panarthropod c-opsin have led to three c-opsins in tardigrades. Although the early-branching nodes are unstable within the metazoans, our findings suggest that the last common ancestor of Bilateria possessed six opsins: two r-opsins, one c-opsin, and three Group 4 opsins, one of which (Go opsin) was lost in the ecdysozoan lineage.

OP-ZS-06

What can we tell students about the body plans of common ancestors of protostomia and deuterostomia and their main subclades

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In introductory lessons to students of biology about comparative anatomy of bilaterian animals we usually use a limited subset of animals which the students have to dissect to get an impression about the organization within animal phyla. Since we have to convey ideas of the change of body plans during evolution it is necessary to show how the organs of animals changed during evolution. Since we got a better knowledge about the course of evolution by use of molecular data we can reevaluate hypotheses about morphological change. Especially it is very probable that within protostomia there was a first large phylogenetic split between lophotrochozoa and ecdysozoa. It seems no longer appropriate to start treating bilateria with plathelminthes as if their organization without an anus, blood vessels and coelomic sacs is ancestral and to treat nematodes after plathelminths so as if they represent a second step of evolution with the invention of an anus which leads to nematodes. It is quite probable that characters like (1) a gut with a mouth and an anus, (2) coelomic sacs with mesenteria and dissepiments enclosing blood vessels and (3) podocytes and meta- and protonephridia were still present in the common ancestor of proto- and deuterostomia. Such an archicoelomate had not necessarily an homonomous segmentation or three pairs of coelomic sacs called proto-, meso- and metacoel. More probable is some serial arrangement. In a similar way we can characterize other architypes.
OP-ZS-07

Nemertean diversity was likely underestimated in the past - the case of the Lineus ruber/viridis - species complex (Nemertea: Heteronemertea)

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Nemertea is a spiralian taxon of unsegmented, soft-bodied, vermiform animals, predominantly inhabiting marine environments. It comprises about 1280 described species of mainly epibenthic predators. The heteronemertean species Lineus ruber (Müller, 1774) and Lineus viridis (Müller, 1774) are among the most abundant and easiest accessible nemerteans at European coasts and therefore the best investigated species, regarding ecology, development, and morphology. However, insufficient original descriptions of L. ruber and L. viridis, exclusively based on external characters, lead to a debate regarding the number and identity of species within the Lineus viridis/ruber-species complex. Concerning morphological data, both species have been synonymized whereas differences in reproduction, development and larval recruitment advocate two species. Different molecular data sets identify from one up to three species. For clarification, we conducted phylogenetic analyses based on mitochondrial 16S and COI and nuclear ITS sequence data from L. viridis and L. ruber specimens collected at different localities of the Northwest Atlantic, English Channel, and North Sea Coasts. We show a division of L. viridis into two clades which are clearly separated from L. ruber. Furthermore, for each cluster we identified an exclusive, albeit rarely encountered morphotype. This study provides an example that diversity in Nemertea might have been underestimated in the past.

OP-ZS-08

Phylogeography of Stygocapitella subterranea (Parergodrilidae) in the Northeast Atlantic.

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Stygocapitella subterranea is a small interstitial annelid inhabiting the upper intertidal of sandy beaches all around the world. However, records from tropical and subtropical regions are still lacking. As is the case for many interstitial species S. subterranea does not possess pelagic larval stages or good active swimming capacities as juveniles or adults. Hence, potential for long-distance dispersal is lacking and, thus, given the global distribution it is very likely that gene flow is limited between these populations. Nonetheless, so far no morphological differences could be found between the different populations. This contradiction is known as the Meiofauna paradox. First molecular analyses based on RAPD data, however, found that gene flow is limited even between populations, which are close to each other, and cryptic speciation occurs by isolation-by-distance. Herein we report new localities for S. subterranea from South Africa, Scotland, Brittany (France) and Sylt as well as sequence data of mitochondrial and nuclear genes from populations in the Northeast Atlantic. The results show that indeed in the Northeast Atlantic cryptic speciation of S. subterranea was not so much driven by isolation-by-distance, but by ecological factors such as grain size. Moreover, population-genetic analyses indicate a rapid spatial expansion of the S. subterranea populations into the Northeast Atlantic, which fits well with retreating ice shelves and newly established seas like the North and Baltic Seas after the maximum of the last glaciations. Hence, the evolution of S. subterranea is more complicated than only being an isolation-by-distance process and other factors such as grain size and glaciations are also relevant.
OP-ZS-09

Reconstructing phylogenies by using shallow sequenced whole genome shotgun data: a case study of a non-model organism

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Recently, multiplexed shotgun sequencing became a powerful tool for reconstructing phylogenies of non-model organisms. Here we present a glycerid phylogeny (Glyceridae, Polychaeta) based on multi-gene analyses. Therefore, we performed whole genome shotgun sequencing for 19 glycerid species and one outgroup species using the Illumina platform. To test the influence of the sequencing depth on the mitochondrial target genes and the nuclear ribosomal cluster we generated artificially reduced datasets from the original datasets comprising 10 million, 4 million and 1 million reads. Filtered sequenced reads of the original datasets as well as of the reduced datasets were assembled de novo by using the assembler IDBA-UD. The assemblies of all datasets were screened for the target genes by BLAST-searches. Significant hits of the original datasets were used to perform a partitioned maximum likelihood analysis in which the monophyly of the Glyceridae is highly supported. Apart from this, all significant hits of the original, as well as of the reduced datasets were used to estimate the abundance of the target genes in each dataset. Therefore, the raw sequenced reads were mapped against the obtained target genes for each dataset by using segemehl. A comparison of the abundances between the datasets gave us a hint how many shotgun reads are at least necessary to reconstruct the same phylogeny like given by the original dataset. This allows for planning a cost-effective strategy to obtain data by multiplex whole genome shotgun sequencing for use in phylogenetic studies of non-model organisms.

OP-ZS-10

Delineating species in population datasets using heterozygosity

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Question:
Molecular approaches to species delimitation based on the Generalized Mixed Yule-Coalescent (including its single-threshold GMYC-ST, multiple-threshold GMYC-MT, and Bayesian bGMYC flavors) are very commonly used to delineate species-level clades of individuals from single-locus sequence data. However, these methods, like the vast majority of tree-based approaches, rely on the assumption that the species to be delineated are monophyletic. By contrast, haplowebs use patterns of shared alleles to group individuals into reproductively isolated species-level taxa that may or may not be monophyletic. What are the relative efficiencies of these two types of approach when dealing with population datasets (comprising many sequences of a relatively small number of species)?

Method:
We conducted a simulation study aimed to compare the performance of GMYC-ST, GMYC-MT, bGMYC and haplowebs under a wide range of population sizes, speciation rates, and mutation rates, for datasets comprised of 1 to 6 species and combining information for 1 to 7 independent loci.

Results & conclusion:
Haplowebs usually outperformed GMYC-based approaches when dealing with population datasets: notably, GMYC-ST appeared prone to overlumping, whereas GMYC-MT and bGMYC presented the reverse bias towards oversplitting (and therefore performed very poorly on monospecific datasets). Although it was originally proposed as a single-locus approach, we found that the performance of bGMYC improved a lot when combining information from several independent loci, and it may therefore represent a good alternative to haplowebs when a large number of independent markers are available.
OP-ZS-11

Except for being pulmonate gastropods, what are the Euconulidae?

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To study ecology and to calculate assemblage metrics, such as taxonomic distinctness between assemblages or regions, it is essential to have a well-established taxonomic and phylogenetic basis. The small snails of the Euconulidae (type genus: Euconulus, originally described from Denmark) are globally distributed. Except for being small, conoidal, brownish, somewhat translucent and usually having some spiral striation, the shells of the Euconulidae seem rather featureless, offering a vast field of options to convergence. It thus is not surprising that many genera that some authors consider to be Euconulidae have been placed in other families by other authors. We thus aim at checking monophyly in this family and identifying genus-level characteristics. In a second step, we aim at testing some biogeographic patterns, including the out-of-Asia hypothesis. If the hypothesis holds true, we expect the earliest branches to be from Asian genera. To this end, we mainly checked the series in the Naturalis collection for potentially mummified specimens. These were photographed with a light microscope and in a SEM, and then subjected to DNA extraction. The following genetic markers were targeted: 16S, 28S, and COI. Here, we illustrate the degree to which shell morphological traits, phylogenetic signals and geographic patterns match.

OP-ZS-12

The impact of reproductive behaviour on salamander phylogeny and evolution

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Obligate neotenic sirenid salamanders are a distinct group within living salamanders. Their breeding biology and in particular their mode of fertilisation ultimately allowing their precise systematic position has long been debated. Observations on external fertilisation and on a rare case of male parental care in one sirenid species (Siren intermedia) have an impact on salamander phylogeny and evolution. Apparently both internal fertilisation and maternal care evolved after the split between phylogenetically more basal salamander groups (Cryptobranchoidea and Sirenoidea) and all other salamanders (suborder Salamandroidea). Amphibians exhibit an extraordinary diversity of reproductive behaviours. Information on these natural history aspects may well help in resolving amphian phylogeny and evolution.
In many cases genitalia are the only attributes allowing to distinguish between closely related species. As one of the driving forces in evolution, sexual selection by female choice seems to have a huge impact on the genital evolution in various animal species, including insects.

In Tettigoniidae males of certain taxa possess a pair of sclerotized genital structures, called titillators that are inserted into the females’ genitalia during mating. During copulation males perform rhythmically movements with their titillators inside the female’s genital chamber. Furthermore, the titillators seem to be necessary to transfer the nuptial gift, called spermatophore, consisting of an ampulla and the spermatophylax.

To get a greater direct benefit, females show a preference for heavier males, producing larger spermatophores. To study the impact of body mass, titillator length and titillator symmetry on the mating success, we conducted mating experiments with the bushcricket *Metrioptera roeselii* (Hagenbach, 1822). Mating is performed in four phases: approach, mounting, copulation and spermatophore transfer.

The results show a significant correlation of male body mass not only with the spermatophore mass but also with the spermatophore transfer duration: heavier males produce larger spermatophores and need less time for spermatophore transfer. Therefore one can assume that heavier males titillate their females more powerful. Additionally, there is a tendency for longer titillators in heavier males.

Moreover, each part of one titillator possesses a certain number of spines. Variability in spine numbers exists between males and within individuals (left-right symmetry) with a great proportion of asymmetric males (50 percent). Whether symmetric males have a higher reproductive success than asymmetric individuals has to be shown in additionally experiments.
PP-BB-02

Multisensory object discrimination in the weakly electric fish *Gnathonemus petersii*

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The weakly electric fish *Gnathonemus petersii* is able to discriminate between a wide range of different objects by using active electrolocation, a process during which the fish perceives object evoked changes in a self generated electrical field. In addition to its electrical sense, these fish also possess a specialized visual system and a mechano-sensitive lateral line system for hydrodynamic perception.

First, we tested whether these fish can also use vision or the lateral line for object discrimination, and second, whether cross-modal transfer is possible. Individual fish were trained to discriminate between two differently-shaped objects of equal volume in a two-alternative forced-choice procedure under three conditions: (i) using all three senses, (ii) using a combination of vision and lateral line or (iii) using only active electrolocation. When they had learned the discrimination task, fish were tested for object discrimination under various combinations of senses available.

All five fish trained without the electric sense were able to discriminate the objects in tests when they could use only active electrolocation. When they were trained with only active electrolocation available, one of the five fish trained was subsequently able to discriminate between the objects visually without the electric sense. These results show that a cross-modal transfer in both directions between vision and the electric sense is possible. When five fish were trained with all three senses available, only one was able to discriminate between the objects using only vision. These results together with the results of the earlier experiments indicate that the electric sense overshadows the other senses during object discrimination.

PP-BB-03

Synchronization of Electrical Discharge Patterns between Weakly Electric Fish (*Mormyrus rume*) and a Mobile Dummy Fish.

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Mormyrid fish produce and perceive weak electric fields, which are emitted as short biphasic pulses from electric organs located in their tails. This unique electro-sensory capability is employed for both active electrolocation and electrocommunication via distinct sensory pathways. Electrocommunication is based on temporal variations of inter-discharge intervals (IDI) which lead to characteristic temporal patterns and frequencies of electric organ discharges (EODs) depending on the behavioral context.

During social interactions, individuals of the weakly electric fish *Mormyrus rume* were found to frequently synchronize their discharge patterns with each other engaging varying individuals within a group.

We replaced communication partners in small groups of freely swimming fish with a mobile dummy fish endowed with the capability to produce arbitrary electric playback patterns based on species specific EOD waveforms. Our results demonstrate that animals synchronize their discharges to playbacks of constant EOD frequencies within their natural occurring frequency range, as well as to naturally occurring discharge patterns that were pre-recorded during well-defined behavioral contexts.

Quantitative analysis of periods where electric discharge patterns are synchronized between a fish and a playback emitting dummy allows the investigation of the significance of electric signaling patterns for electrocommunication during social encounters.
PP-BB-04

Spatial orientation strategies in the weakly electric fish, Gnathonemus petersii

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Spatial orientation is an essential task for all mobile animals. Two main strategies have been identified for animal navigation in an environment: (i) egocentric orientation - a self-referred strategy and (ii) allocentric orientation - a landmark-referred strategy. Here we tested which orientation strategy the weakly electric fish Gnathonemus petersii uses during navigation through a maze containing two barriers with two alternative doors, each. The fish were trained individually to swim through a certain combination of doors (first decision = right, second decision = left), which were marked by objects placed in front of them. During training, the alternative door of each barrier was closed, respectively. After the fish had learned to swim through this maze on a direct path, it was tested which route the fish used when all doors were opened (i) with the objects remaining in front of the trained doors and (ii) when the objects were placed in front of the alternative doors. The results show that all four trained fish had learned the swimming direction at the first barrier, because they were still swimming through the trained door (right) when all doors were opened. They also stuck to this direction when the object was placed in front of the alternative door, suggesting the use of an egocentric orientation strategy. At the second barrier, the fish tended to swim not through the trained left door but again to the right one bringing them at a straight path to their goal. This might be explained by the fact that they could see the now open door and used the shortest direction to their goal. In future experiments, we will investigate, whether the senses available during route learning have an influence on the orientation strategy used.

PP-BB-05

Influence of arbuscular mycorrhiza on performance and behaviour of a generalist herbivore

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Recently it has been shown that the symbiosis between plant roots and arbuscular mycorrhizal fungi leads to pronounced changes in the plant leaf metabolome. These changes are plant species-specific and can have direct or indirect effects on the performance of herbivorous insects. Especially generalist chewing herbivores should be strongly influenced by changes in the plant chemical composition. As a consequence of different performances, the behavioural phenotypes may be also affected. Therefore, we tested whether the performance as well as the behaviour of a generalist herbivore, Heliothis virescens (Lepidoptera: Noctuidae), differ between caterpillars reared on mycorrhizal (AM) and non-mycorrhizal (NM) plants of Platago major. A battery of behavioural traits was tested repeatedly over the development to determine whether behaviours are expressed consistently over context and time. Mycorrhization of the plants had only little impact on the performance and behavioural dimensions such as activity and exploration. Furthermore, the experience of the caterpillars did not induce a feeding preference for NM versus AM plants. We conclude from our results that the herbivores show a high plasticity in response to AM-mediated changes in plant chemistry within a given plant species but may show more pronounced differences when feeding on host species of more distinct chemistry.
PP-BB-06

Experimental infection of a periodical cicada (*Magicicada cassini*) with a parasitoid (*Emblemasoma auditrix*) of a proto-periodical cicada (*Okanagana rimosae*)

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The protoperiodical cicada *Okanagana rimosae* is subject to infection by the acoustically orientating parasitoid fly *Emblemasoma auditrix*. Here we test the question, whether the parasitoid can also infect periodical cicadas (*Magicicada cassini*) and which steps of the parasitation process can be completed. The hearing threshold of *E. auditrix* matches with the spectrum of the calling song of *M. cassini*. Behaviourally, host localization is possible by the parasitoid as it approaches a loudspeaker broadcasting the calling song of *M. cassini*. *M. cassini* is readily accepted as host and for host infection the parasitoid uses the same behavioural sequence as for its host *O. rimosae*. A larva is deposited into the timbal of the cicada. By contrast to *O. rimosae* the development of the fly larva is delayed and eventually suppressed in *M. cassini*. These experimental data are discussed in the view of the evolution of the periodicity in the periodical cicadas.

PP-BB-07

The impact of long-term alarm cue exposition on attractiveness and mate choice decisions in a cichlid fish

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Plastic antipredator adaptations provide clear survival advantages to prey animals in the presence of predators. However, such phenotypes favoured by natural selection may face costs during sexual selection as a predator-adapted phenotype might be less attractive. Furthermore, mate choice decisions themselves might be influenced by predation as the optimal mate partner in a predator environment might differ from the optimum in a predator-free environment. In our study, we used the West African river cichlid *Pelvicachromis taeniatus* as a model system to address these hypotheses. Fish in our experiment were raised in a split-clutch design under simulated presence or absence of predation risk until reaching sexual maturity. To achieve this, fish were either supplied daily with a dose of distilled water or chemical alarm cues from injured conspecifics which reliably signal predation. In a dichotomous mate choice setup, we gave adult fish the choice to court with two size-matched potential mates of the opposite sex; one mate was derived from the predation treatment whereas the other one was derived from the control treatment of the same clutch. As *P. taeniatus* shows mutual mate choice, we aimed to investigate the impact of predation risk on mate choice in both sexes from both treatment groups. The results revealed that both males and females from the predator-free environment preferred predator-adapted mates. In contrast, focal fish from the predator treatment indiscriminately selected a mate. Our study suggests that not only natural but also sexual selection acts on predator-adapted phenotypes in *P. taeniatus*. 
**PP-BB-08**

The ontogenetic significance of animal play: developmental costs and benefits

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The developmental costs and benefits of physically active play in infants are a puzzling topic in biology, psychology and health sciences. The most prominent hypotheses propose that investment in play accelerates motor skill acquisition but requires that surplus energy is available after the fixed investment in growth, i.e. growth takes ontogenetic priority over play. However these hypotheses remain largely untested. We investigated the relationships between growth, play and motor skill acquisition and studied all 17 infants of an unprovisioned wild group of Assamese macaques (Macaca assamensis) living in their natural habitat at the Phu Khieo Wildlife Sanctuary in Thailand. We combined behavioral observations on social play and latencies of motor skill acquisition with quantitative measures of natural food availability and individual growth rates measured noninvasively via photogrammetry. We found that physically active social play causally accelerates motor skill acquisition. In contrast to current theory our results emphasize that play carries developmental costs because even under moderate energy restriction, investments in physically active social play were strongly at the expense of growth with consequences for life history. As part of this energy tradeoff male immatures invested more in social play for motor skill acquisition at the expense of growth. Our results show that depending on skill needs, play can be of equal or higher ontogenetic priority than unconstrained physical development, which underscores the ontogenetic and evolutionary significance of play.

**PP-BB-09**

Does visual lateralization affect female mate choice and male-male aggression in three-spined sticklebacks (Gasterosteus aculeatus)?

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Lateralization i.e. the differential use of the left and right hemisphere for specific cognitive, perceptual and behavioural tasks has been demonstrated in a variety of vertebrate taxa. Brain lateralization is suggested to be beneficial during simultaneous processing of multiple stimuli. Behavioural asymmetries in lower vertebrates can be studied by measuring preferences in eye use since most species do not have overlapping optical fields and visual information is transferred from each eye to the contralateral hemisphere. While in fishes visual lateralization was described for different intraspecific interactions such as shoaling behaviour and individual recognition, its role during inter- and intrasexual interactions is largely unexplored. In the present study, we asked whether behavioural lateralization influences female mate-choice decisions and male-male aggression in a fish, the three-spined stickleback (Gasterosteus aculeatus). In a first experiment, gravid females were given the choice between two computer-animated males differing only in the intensity of their red breeding coloration. Mating preferences of females were tested for three eye conditions: left eye occluded, right eye occluded, both eyes open. By using the same eye treatment, aggressive behaviour of reproductively active males was quantified in mirror-image trials in a second experiment. The results will be discussed in the context of potential proximate mechanisms underlying mating preferences and intrasexual competition.
PP-BB-10

Underwater sound localization of pure tones in the median plane by harbor seals (*Phoca vitulina*)

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In an underwater environment the physical characteristics of sound propagation differ considerably from those in air. Thus sound localization underwater is associated with great difficulties, especially in the median plane. For terrestrial mammals monaural cues are given through reflection and diffraction at the head and especially at the external ear, relying on impedance differences between pinna and air. Underwater this difference is negligible because of the similar density of soft body tissue and water. Furthermore all phocid seals lack an external ear.

The approach of the present study was to investigate whether harbor seals (*Phoca vitulina*) are able to determine the direction of an acoustic signal in the median plane despite these difficulties. Testing was conducted with four male harbor seals in a semi-circle area of 6m in diameter in about 2.5 m depth. The seals’ performance was determined for sine wave stimuli from 350 Hz to 16 kHz using a two alternative forced choice method to measure the minimum audible angle (MAA) for each subject and each frequency.

For the first time it was shown that harbor seals are able to localize a pure tone in the median plane with a high performance for low frequency stimuli between 350 Hz and 2 kHz with MAAs ranging from below 2.5° up to about 25°. For higher frequencies the animals show strong individual differences. These results show that harbor seals have the ability to localize at least low frequency signals in the median plane, but the sensory mechanism underlying still has to be clarified.

PP-BB-11

Anemotactic orientation in leaf-cutting ants during digging

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The grass-cutting ant *Atta vollenweideri* inhabits giant underground nests and builds large conical-shaped mounds uncovered of vegetation. A mature nest may contain up to 200 nest openings, most of them very large, through which air moves to allow a continuous gas exchange between the nest and the environment. The direction of the air-flow depends on the location of the nest openings, i.e. the central ones serve as outflow conduits, whereas the peripheral ones serve as inflow conduits. To enhance nest ventilation, workers build conspicuous turrets on top of the nest mound. Since workers build ventilation turrets mostly on outflow conduits, we hypothesized that ants are able to perceive the direction of air movements.

In the laboratory, workers of *Atta vollenweideri* that experienced either inflow or outflow of air while digging were subsequently given a choice between two alternative digging sites in a T-maze, one providing inflow and the second one outflow of air. Ants that previously experienced an inflow of air significantly chose the inflow site to continue digging, whereas workers that experienced an outflow of air preferred the outflow site. These results indicate that ants are able to perceive the direction of air currents inside the nest and can use them as orientation cues during nest digging.
**PP-BB-12**

**Multidimensional decision-making in a small circuit**

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The vast majority of investigations in understanding the neural groundwork of decision-making are confined to tasks with only two alternatives even though many common decisions normally consist of more than two alternatives. Hunting archerfish for example are not only famous for shooting down aerial prey objects, but also for their astonishing skill to predict accurately the later impact point of their still falling prey. Three independent variables, namely the prey's initial speed, starting height and direction, have to be detected and combined for this prediction and the following decision - startle response or not. So far, all experiments were carried out with a horizontal launched prey. Here we ask, what will happen if the prey follows an additionally vertical motion? In this case the fish would have to estimate an additive vertical upward component, adding a fourth dimension. Failing to account for this information would be detectable experimentally from predictable errors the fish would make, e.g. archerfish will miss the correct point of impact or fail in arriving there just in time with the landing prey. In this study we show for the first time, that archerfish have the ability to cope with an additional vertical motion. Hence, archerfish not only master decision-making based on three independent variables, but also based on four variables without an appreciable decline in accuracy or timing.

**PP-BB-13**

**Learning and recall of a spatial memory task in weakly electric fish (Gnathonemus petersii)**

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The present study focuses on the learning and recall of a spatial memory task in the weakly electric fish *Gnathonemus petersii*. We analyzed the performance of fishes in a spatial memory task, where fish had to find a food rewarded location within a circular arena.

In general fish are known to be able to use the geometry of the surrounding environment and the spatial arrangement of cues (landmarks) for orientation, a skill called “spatial orientation”. To analyze the position of a goal relative to the landmarks, animals need a “cognitive map” which provides a representation of the environment. Such a representation would allow the animal to find its way irrespective of its starting point in a known area. Presently knowledge about spatial memory strategies of weakly electric fish is scarce.

*G. petersii* (N=10) were trained to find food in total darkness in one of fifteen feeders and videotaped using IR illumination. The feeder was always in the same place during training and was additionally marked by a spatial cue (metal pyramid). After two consecutive successful training sessions (three correct choices out of ten trials) a test session was conducted. This either was another rewarded session (recall) or an unrewarded session without the local cue (relearning/extinction).

All fish performed the spatial orientation task successfully. We will analyze the number of sessions until attainment of the learning criterion, average time until choice, number of errors, swimming trajectories and distance covered over trials. To find out more about the animals’ learning strategies we will study the results of trainings and test sessions of the learning groups.
Behavioral Biology

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PP-BB-14

Timing in pinnipeds

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Time along with space is one of the two fundamental dimensions of life. Whereas spatial aspects have already been considered in experiments with marine mammals, research has not focused on timing abilities so far. However, living in the marine environment might require a precise sense of time as e.g. tides control access to food as well as haul-out places. Moreover, a precise sense of time would be beneficial for orientation, e.g. to integrate a homing vector.

The aim of the present study was to investigate if harbour seals (Phoca vitulina) and South African fur seals (Arctocephalus pusillus) possess a sense of time and how sensitive they are in judging time intervals. Experiments were conducted under constant conditions inside an experimental chamber. In a two-alternative-forced-choice procedure, the two experimental animals were required to discriminate between standard and longer comparison time intervals marked by a visual stimulus that was displayed on a monitor. Time difference thresholds were determined for five standard time intervals (3s, 5s, 7s, 12s, 18s) by a staircase method. Both seals learned the task easily and they were able to discriminate time intervals in the millisecond to second range.

In conclusion, seals possess a well-developed sense of time which is most likely fundamental for successful orientation in their environment.

PP-BB-15

Infant care in saddleback tamarins (Saguinus nigrifrons)

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In cooperative breeding systems breeding is usually restricted to one female per group and infant care is shared by group members (mother and helpers). Exceptions to single-female breeding are associated with reduced infant survival. Therefore multiple breeding situations raise the question of how infant care is allocated by helpers. This question is particularly relevant in marmosets and tamarins where twin’s neonatal body mass corresponds roughly to 15-20% of maternal body mass, making infant carrying a costly behavior. In small groups living Neotropical tamarins (Saguinus) polyandry is the prevailing mating system and adult males are the principal helpers.

We therefore addressed the question of how infant care (carrying and feeding) is allocated by helpers and mothers in groups with single-female and multiple-female breeding. We conducted a study on 5 wild habituated groups of saddleback tamarins (Saguinus nigrifrons) at the Estación Biológica Quebrada Blanco in north-eastern Peru in 2012 and 2013. Data collection focused on social and sexual interactions; additionally fecal samples for hormonal (testosterone, progestogen, cortisol) and genetic analyses (kinship and paternities) were collected. Here we report patterns of infant care of saddleback tamarins in general and in a multi-female breeding context. Furthermore we address possible costs and benefits for group members to participate in infant care. Results indicate that mothers need to compensate for reduced infant care by males in a multi-female breeding context. This might be explained by physical constraints on males’ care for a second set of infants or by uncertainty of paternity.
**Size discrimination of single vortex rings by stationary harbour seals (Phoca vitulina)**

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While hunting in dark and turbid waters seals often have to deal with limited vision. Harbour seals (Phoca vitulina) e.g. cope with this problem by possessing vibrissae (whiskers) highly sensitive to water disturbances. These hydrodynamic sensors allow the animals to detect and track hydrodynamic trails left behind by prey fish. These trails differ considerably depending on the fish species, the fish’s body shape, size, and swimming style. Therefore, the trail may reveal information about its generator. Most of these trails consist of a chain of linked vortex rings but there are diverse natural situations in which single vortex rings may be encountered, e.g. during a c-start escape response of some fish species. Even those single vortex rings might contain useful information for a harbour seal. In this study, the ability of two blindfolded stationary harbour seals to perceive and discriminate the size of two single vortex rings has been investigated. In an experimental pool two vortex ring generators were fixed on either side of a point directly ahead of the stationary seal. Single vortex rings with predefined sizes, velocities, and accelerations similar to those produced by fish were generated consecutively. In every trial one of the generators produced a bigger vortex ring travelling to the seal’s vibrissae than the other generator. The animal had to distinguish the bigger vortex ring, either coming from the right or the left side. Preliminary results of one animal show a highly significant performance for three tested stimulus combinations. This ability could be advantageous for harbour seals to gain information about a prey item when hunting fish even if no complex hydrodynamic trail is available but instead only single vortex rings.

**Feeding competition in female Assamese macaques and the role of conflict avoidance**

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The quality, availability, and distribution of food resources and their influence on types and levels of feeding competition play a central role in ecological models of female social structure in mammals. Here we discuss the impact of social and ecological factors on energy intake, rates of food related aggression and the use of competition avoidance mechanisms in female Assamese macaques (Macaca assamensis). Data were collected on one group of wild Assamese macaques at Phu Khieo Wildlife Sanctuary in north-eastern Thailand during two one-year periods (October 2007- September 2008, Mai 2010-April 2011). Over 2100 hours of focal animal data (including all adult females: N=12, N=15) were collected in addition to 354 observations of feeding competition at the level of individual food patches, 84 group scans of individual spatial position, information on the nutritional content of important food items, monthly phenology data (650 trees, shrubs and climbers) and information on food tree size and density (54 botanical plots, 20.75 ha: 11,508 trees and climbers). Female dominance rank does not affect energy intake or activity and these relationships are not affected by food abundance, density, quality or size. The frequency of aggression in food patches increases with increasing feeding group size and decreasing patch size. The absence of a rank related skew in energy intake may be explained by competition avoidance mechanisms females employ, including the use of alternative feeding sites within food patches, storing food in cheek pouches, and co-feeding with individuals they share strong social bonds with. Additionally, lower ranking females appear to avoid feeding competition by occupying more peripheral positions within the group which may increase their predation risk.
The epithelial microfilament pattern of *Drosophila* ovarian follicles: dependence on intracellular pH and membrane potential

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The follicular epithelium of *Drosophila melanogaster* is an appropriate system for studying epithelial development. During the course of oogenesis, the epithelium differentiates into several morphologically distinct follicle-cell populations. Changes in cell morphology are reflected in rearrangements of the actin cytoskeleton. During early oogenesis, microfilament bundles contacting the basement membrane are oriented perpendicular to the long axis of the follicle. Later on, this preferred orientation disappears gradually towards the posterior pole while it is maintained in follicle cells migrating centripetally between nurse cells and oocyte. Since bioelectric phenomena are known to affect developmental processes as well as cytoskeletal dynamics, we analysed the influences of intracellular pH (pH$_i$) and membrane potential (V$_{mem}$) on the orientation of epithelial microfilament bundles. Using the fluorescent pH-indicator 5-CFDA,AM and the potentiometric dye DiBAC$_{4}$, we found stage-specific bioelectric patterns within the follicular epithelium: Both apicobasal gradients of pH$_i$ and V$_{mem}$ as well as differences between specialized follicle-cell populations were observed. Manipulating pH$_i$ and V$_{mem}$ by the inhibition of ion channels and transporters (V-ATPases, Na$^+$/H$^+$-exchangers, ATP-sensitive K$^+$-channels, L-type Ca$^{2+}$-channels) resulted in modifications of the epithelial microfilament pattern. The inhibitory effects ranged from altered preferred orientation to complete loss of the parallel bundle arrangement in individual cells. Thus, electrochemical cues, like pH$_i$ and V$_{mem}$, seem to be involved in organizing the microfilament pattern and in shaping the follicular epithelium as well as the whole follicle.

FGFR signaling in Hydra: docking proteins and other downstream signaling elements

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FGFR signaling is conserved in animals at a level which allows a heterologous rescue of *Drosophila* mutant by a Hydra FGFR (1, 2). FGFR signalling in *Hydra* is tightly coupled to boundary formation and bud detachment (2, 3, 4). We asked whether downstream signalling elements required to dock FGFR to diverse signalling elements are conserved in the polyps. This question is interesting because vertebrates and Drosophila use completely unrelated docking proteins. FRS2 serves this function in vertebrates, Dof/stumps in Drosophila. We therefore screened the genomic and EST databases (5, 6, 7) for these typical FGFR docking proteins and used expression profiling by in situ hybridization to localize them in the tissue.

We found both proteins being encoded and transcribed in the hydrozoan *Hydra* and the anthozoan *Nematostella*. Expression analysis in *Hydra* failed to detect FRS2 at high levels in regions where the two Hydra FGFRs are expressed. Similarly, Dof expression could not be directly related to regions of high FGFR expression. We discuss these findings under the aspects of dynamic tissue movement in Hydra and the possibility that zones of high expression do not necessarily correspond to zones of high protein content.

(1) Rudolf et al., 2013; (2) Rebscher et al., 2010
(3) Sudhop et al., 2004; (3) Münnder et al., 2010; (4) Böttger and Hassel, 2013;

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PP-DB-03

Functional analysis of Sp family genes in insects and spiders

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One of the largest families of DNA binding proteins are zinc finger transcription factors, often involved in transcriptional regulation. The Sp-family of transcription factors are evolutionary conserved zinc finger proteins found in a variety of metazoan species. Previous work showed that the Sp-family genes can be grouped into three different clades, namely Sp1-4, Sp5/Btd and Sp6-9. All members of this Sp-family share three highly conserved Cys2His2-type zinc fingers, which bind to G-rich DNA elements and a conserved btd box. Many control regions of tissue-specific as well as ubiquitously expressed genes contain these binding sites, indicating that Sp-family transcription factors have the potential to regulate a large number of target genes. Findings from the fruit fly Drosophila melanogaster (Dm) indicate that CG5669, a member of the Sp1-4 clade, is involved in cell differentiation and cancer. buttonhead (btd), a member of the Sp5/btd clade, is involved in the formation of several head segments in Dm, but not in Tribolium castaneum (Tc). Genes from the Sp6-9 clade are involved in allometric limb growth in Dm, Tc and the milkweed bug Oncopeltus fasciatus (Of). We will study Sp-genes of all clusters in two additional arthropod species, namely Of and the common house spider Parasteatoda tepidariorum (Pt), to get new insights in conservation of Sp genes and the functional changes of btd during the arthropod evolution.

PP-DB-04

Developmental genetic studies of the embryonic development and metamorphosis of the pedipalp using the spider Parasteatoda tepidariorum (C. L. Koch, 1841)

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The spider pedipalp is a multifunctional appendage used e.g. for feeding, sensing and mating. It therefore has an important role in adaptation and speciation in spiders. The genetic basis of pedipalp development and metamorphosis, however, is not well understood. Thus, the contribution of developmental mechanisms to the evolution of pedipalp morphology and functional diversification and adaptation cannot currently be appreciated. We aim to identify genes that are required for pedipalp formation in the embryo and pedipalp metamorphosis in the post-embryonic stages in the model spider species Parasteatoda tepidariorum. Using two available RNA interference (RNAi) mediated phenotypes that lead to embryonic pedipalp loss and embryonic pedipalp duplication, and differential gene expression analysis of embryonic transcriptomes, we aim to identify the gene set required for embryonic pedipalp formation. We also investigate the postembryonic contribution to pedipalp morphology. Analysis of differential gene expression in male pedipalp tissue before, during, and after metamorphosis will identify genes controlling pedipalp metamorphosis. The function of these genes will be tested via a modified technique for postembryonic RNAi to affect only the metamorphosis of the pedipalp. Genes resulting in an especially interesting RNAi phenotype in Parasteatoda tepidariorum will also be isolated and studied in Parasteatoda lunata, a closely related spider species that, however, differs in pedipalp morphology, to provide a comparative perspective.
PP-DB-05

Gene expression during body appendage development in caudal regenerates of the polychaete annelid Platynereis dumerilii

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The formation of body appendages is regulated by a gene-network of partially overlapping subset of genes shared by different taxa. The function of some genes appears to be more basic, like the proximodistal patterning of the appendage. Other genes rather regulate the development of taxon-specific differences. However, our knowledge on formation of appendages in trochozoa is still limited due to the limited number of such structures and to the publications with molecular data. The polychaete P. dumerilii features various types of appendages, including parapodia and anal cirri. These animals are also capable of regenerating both the pygidium as well as posterior segments after amputation of substantial parts of the posterior trunk. Caudal regeneration forms the pygidium and new segments at a rapid pace allowing a sequential observation of temporal and spatial gene expression in forming appendages in a single posterior end.

We isolated sequences of five genes involved in appendage formation in other taxa from P. dumerilii: homothorax, extradenticle, distal-less, decapentaplegic and buttonhead. Analyses of expression patterns during caudal regeneration by in situ hybridization reveal striking similarities related to expression in arthropods and vertebrates. All genes exhibit transient expression during maturation of segments. As shown in other phyla, Pdu-hth and Pdu-exd are co-expressed, expression yet not being limited to proximal part of the parapodia. Pdu-dll is also upregulated in the anal cirri. The patterns observed suggest conserved roles of the previously mentioned genes during appendage formation. The underlying mechanisms utilizing these genes during formation of body appendages might yet not be identical across taxa.

PP-E-02

Fungal allelopathy suppresses anti-fungivore defence in a mycotoxigenic mould

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Invertebrate grazing is an important factor affecting fungal physiology, growth and anti-fungivore defence. Fungivory has also been shown to influence fungus-fungus interactions, however, the reverse, how fungal competition regulates anti-fungivore properties and thereby determines invertebrate success remains largely unknown. We demonstrate, for a tripartite fungal-insect model system, that habitat-specific release of allelopathic metabolites by the yeast Saccharomyces cerevisiae strongly affects morphological and chemical differentiation of the mycotoxigenic mould Aspergillus nidulans. This phenotypic shift leads to a consistently reduced capacity to harm fungivorous Drosophila melanogaster larvae and impairment of the inducibility of genes involved in resistance to insect grazing. Our study supports the notion that the shape of trophic links can be substantially altered by fungal allelopathy and confirms that heterogeneity in the outcome of fungus-fungivore interactions is linked to variation in inducible anti-fungivore defence.
Odour-based location of mutualist host and toxic non-host microfungi by *Drosophila* larvae

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In analogy to herbivore-plant interactions, fungivorous animals may find suitable host fungi by exploiting fungal borne volatiles and use such chemical information to distinguish between fungi of varying profitability. We hypothesize that *D. melanogaster* larvae are able to discriminate between host (mutualist yeast) and non-host (toxic mould) fungi, and that fungus-specific variation in the emission of volatile organic compounds influences larval foraging decisions. By observing larval behaviour, the yeast *Candida californica* was found to be more attractive than the mould *Penicillium expansum*, yet the mould did not repel larvae. By means of SPME-GC-MS, we identified four yeast-specific and seven mould-specific volatiles. Two alcohols were emitted by both *P. expansum* and *C. californica*. Time-dependent behavioural response profiles of ~600 individual *D. melanogaster* larvae revealed attraction to yeast-specific organic acids but indiscriminate attraction to mould and yeast alcohols. Mould-specific compounds (e.g. terpenoids) neither attracted nor repelled larvae. We therefore suppose that *D. melanogaster* larvae base their foraging decisions on quantitative differences in the emission of unspecific fungal volatiles. The inability to perceive mould-specific compounds may provide a functional explanation for the general attraction to host and non-host fungi in *Drosophila* larvae.

Poison is a matter of perspective: The Lily of the valley (*Convallaria majalis*) and the Onion Beetle (*Lilioceris merdigera*, Chrysomelidae, Criocerinae)

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Elected the „Poisonous Plant of the Year 2014“ in Germany, the Lily of the valley *Convallaria majalis* owes its toxicity to the production of over 35 different glycosides belonging to the group of so called cardenolides. Cardenolides inhibit the ubiquitous and essential enzyme Na/K-ATPase, which is found in the plasma membrane of all animal cells. Nevertheless, larvae and imagines of the Onion Beetle *Lilioceris merdigera*, which are often found on *Allium sp.*, also feed on the leaves of *C. majalis*. After having fed on *C. majalis* for >5 days followed by *Allium* for 3 days to ensure a cardenolide-free gut content, neither the defensive secretions of *L. merdigera* nor the beetles themselves contained any cardenolides. Sequence analysis of the beetle’s Na/K-ATPase revealed none of the mutations known to reduce the enzyme’s sensitivity to cardenolides in other species. In tracer feeding experiments with the \(^3\)H-marked cardenolides ouabain and digoxin, the bulk of the radiation was recovered from the beetles’ feces, indicating a protective mechanism in the gut wall that prevents cardenolides from entering the hemolymph. Disposing of cardenolides via feces may be advantageous for the larvae of *L. merdigera*. They cover themselves with a fecal shield, which often exceeds the size of the larva itself. The fecal shield not only provides optical camouflage, as has been shown in other chrysomelid larvae (subfamily Cassidinae), but, containing cardenolides, it may additionally provide chemical protection as we assessed in bio-assays with predatory ants.
PP-E-05

Direct effects of sublethal ambient UVB-radiation on dorsal body coloration and activity of three-spined sticklebacks (Gasterosteus aculeatus)

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Ultraviolet radiation (UVR), especially the UVB range (280-320 nm), represents a strong ecological stressor for aquatic organisms causing effects and responses on cellular, molecular, morphological and behavioral levels. In fish, it can be assumed that melanophores have a protective feature by absorbing UVR and neutralizing reactive oxygen species (ROS). In contrast to UVA, UVB has an enhanced cytocidal effect due to the higher amount of energy, which possibly counteracts formation of melanophores. Direct effects of UVB may also be found on the behavioral level. The most effective behavioral response to harmful UVB radiation would be avoidance of areas of intense radiation. In the present study we asked whether UVB-exposure has direct effects on dorsal pigmentation and swimming activity of three-spined sticklebacks (Gasterosteus aculeatus). A first experiment in the laboratory dealt with the effects of UVR on activity by comparing swimming activity of fish during UVB-exposure to a control treatment providing only visible light. A further experiment should examine the change of dorsal coloration (total brightness) of sticklebacks during four weeks of enhanced UVB radiation in relation to a control group without additional UVB. In this experiment, fish were kept in outdoor mesocosms under semi-natural conditions. Sublethal amounts of UVB radiation were used in both experiments. The results showed an increased general activity during UVB-exposure compared to control fish and an increase in dorsal brightness in UVB-exposed three-spined sticklebacks relative to control individuals.

PP-E-06

Microsatellite genetic variability between populations of the coconut crab Birgus latro (Decapoda: Anomura: Coenobitidae) from Christmas Island in the Indian Ocean

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The coconut crab Birgus latro is the largest terrestrial arthropod and has a marine planktonic larval phase of approx. up to 4 weeks. Species with marine planktonic larvae have long been thought of having the potential for long-distance dispersal and being demographically open. New evidence, however, increasingly points at a lower degree of population connectivity and a higher degree of self-recruitment. Along with the Aldabra Atoll (Seychelles) and the Chagos Archipelago, Christmas Island is one of the few islands in the Indian Ocean still populated by B. latro. The population on Christmas Island has already been shown to be clearly genetically divergent from populations in the Pacific Ocean. The island in the Indian Ocean nearest to Christmas Island and harbouring a small population of B. latro are the Cocos Keeling Islands, approx. 900 km to the west. The mostly protected population on Christmas Island is reported to be the world’s largest and comparatively stable, but has experienced dramatic losses during the past 100 years, due to habitat loss by phosphate mining and road kills. Even though the island only comprises 135 km², it is structured by staggered plateaus separated by rugged cliffs, and holds several different habitats. Females of B. latro, and most likely also males, undergo reproductive migrations, but the distances covered are not known, and the crabs also exhibit site fidelity. Here, we present findings on the degree and pattern of population subdivision and population structure of B. latro on Christmas Island, using microsatellite markers.
PP-E-07

RFID-reader is watching you: measuring activity patterns in free-living edible dormice (Glis glis)

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Fluctuating environments force mammals to adjust their physiology and behaviour to changing conditions. The edible dormouse (Glis glis), a small nocturnal hibernating rodent, is able to adjust its behaviour to cope with these challenges of changing climatic conditions and “pulsed resources”. Early in spring, edible dormice can anticipate upcoming seed availability in late summer and adjust their reproduction accordingly. A high percentage of the population only reproduces in years with intermediate or high masting of beech trees, which provides juveniles with high caloric food in July/August and increases their survival probability over winter. Between 2006 and 2013 we studied dispersal rates of edible dormice at our study site in the Vienna Woods. Our results show that mainly male juvenile dormice disperse over longer distances, whereas especially older females show a very small propensity to leave their territory, in particular if their territories contain old beech trees, which have a higher tendency to fructify. Even in good habitats, minimizing of foraging time is crucial for dormice, because predation pressure (e.g. owls, martens) and hence mortality risk increases with the time spend foraging. We used microprocessor-controlled RFID-readers in the field, installed at the entrances of nestboxes, to measure times of departure from and return to the nest during the night. Additionally, faecal samples were taken to measure glucocorticoid metabolites in the animals, to investigate the influences of foraging time and activity patterns on stress hormone levels and reproductive success of edible dormice.

PP-E-08

Ubiquitous eight-carbon volatiles of fungi are infochemicals for a specialist fungivore

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Fungal substrates release an enormous variety of volatile substances. Fungal oxylipins have recently gained attention as signalling molecules of fungal kingdom and cross-kingdom communication. The eight-carbon volatiles - their volatile breakdown products - are the dominant volatiles in most fungal taxa. They have been repeatedly shown to attract fungivores. Here we identified eight-carbon volatiles that elicit a more differentiated behavioural response of the fungivore Bolitophagus reticulatus.

Gas chromatographic methods were used to identify eight-carbon volatiles of its host fungus Fomes fomentarius and to trace individual release of 1-octen-3-ol, 3-octanone and 3-octanol over successional stages of fruiting bodies during beetle colonisation. Olfactometer bioassays were used to test the behavioural effect of these compounds to foraging beetles; electroantennography was used to characterise their antennal responses.

Nine eight-carbon volatiles have been identified. 3-octanone was attractant, 1-octen-3-ol repellent and 3-octanol indifferent in behavioural bioassays. Concentrations are considerably different among successional stages of fruiting bodies and correlate to beetle abundance in the field, indicating their infochemical function.

Our results show that a specialist fungivore is able to differentiate the most common eight-carbon volatiles of mushrooms to assess host quality. Key roles and marked similarities of fungal to plant oxylipins suggest a comparable importance of eight-carbon volatiles to fungivores as green-leaf volatiles (GLVs) have to herbivores.
PP-E-09

Can phylogenetic diversity serve as a proxy for functional diversity of soil-living oribatid mites in different land-use types?

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Traditional diversity indices cannot cover all aspects of biodiversity, leading to misconceptions on value and role of species and communities in different environments. Functional diversity is a better predictor of a species’ role in ecosystems, however it is difficult to measure for all species present in a study site. If functional traits are conserved within a clade of species, phylogenetic diversity can serve as an easy applicable tool to measure the functions of species in an ecosystem. Many studies in terrestrial habitats have successfully applied this approach on plants and aboveground animals, showing that land-use type has an effect on species, phylogenetic and functional composition. The soil, however is a vacant field still to be tested and soil animal communities have shown different trait values suggesting different functions in habitats under human activities. In this study we use data of soil-living oribatid mites of four forest types in three regions across Germany and check if (i) phylogenetic distance is able to describe functional distinctness and (ii) functional distinctness correctly explain oribatid mite composition in different soil habitats. Our study will give new insights into the effect of land-use on biodiversity and community processes of belowground systems.

PP-EB-01

Covariation of Skull and Dentition in Domestic Dogs - A Geometric Morphometric Study

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Domestic dogs show a remarkable variation in skull size and shape which developed over a relatively short time span and exceeds that found in wild canids. Specifically dog skulls differ significantly in rostrum length and shape as well as in cranial flexion. Yet, little is known how these rapid transformations in cranial morphology among modern dog breeds have also affected the size and shape of the mandible and the dental arches. Here we used geometric morphometrics to test for covariation among the dento-cranial modules in various dog breeds. To this end, we used CT images of skulls of 130 adult dogs (102 breeds) and defined 99 three-dimensional landmarks subdivided into four subsets representing the cranium, mandible, and upper and lower dentitions. Principle component analyses of Procrustes fitted shape coordinates were used to identify the main shape change variables in the skull. Partial least square analyses were employed to analyze covariation patterns. We show that length, ‘massiveness’ and flexion of the cranium explain the shape variables creating most of the variation in dentition and mandible. The upper tooth row co-varied with cranial shape by varying curvature, relative size and orientation of incisors (procumbence). Lower tooth row shape was highly correlated to upper tooth row and cranium and displayed similar shape variation. Mandible shape, which exhibited the lowest correlations with other modules, covaries with shorter snouts by upwards bending and increasing relative mandible body length. We conclude that shape changes of the cranium have direct influence only on upper tooth row shape. However, proper occlusion mediates cranial shape changes to the lower tooth row and mandible.
PP-EB-02

Sensory evolution in tettigoniids: how communication shape the auditory system

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Acoustic communication is very important for mate attraction in Tettigoniidae. In most species of this group, males produce calling songs which are detected by conspecific females with tympanal hearing organs located in the proximal tibia.

In some groups the communication system is bidirectional and shows a duet of the initial male calling song and the female reply. Only the male or both individuals approach for mating. The sensory ecology of duet signalling enforces sex-specific adaptations: male calling songs are usually louder than female replies. This fact leads to the hypothesis that male ears in duetting species should be more sensitive for detection of the female's reply.

We investigated the effect of the communication system on the hearing organ in European tettigoniids of the genus *Poecilimon*. This genus is an ideal study system as most species use duetting signals, while females lost in two groups the possibility for a reply. In these cases the communication system is based on male songs only.

To estimate adaptive morphology of the auditory organ and possible correlation to the communication system, we measured the sizes of sound-transmitting spiracles and tympana in representative species of both communication systems. In addition, the number of auditory sensilla was compared between species.

Our findings indicate a sex-specific adaptation of the auditory system related to the role of female signalling. We discuss evidence for regression of hearing in species which have lost the females acoustic response.

PP-EB-03

Parenthood analysis and population assignment in wild boar (*Sus scrofa*): How many microsatellites to use?

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Microsatellites are regularly used to identify individuals, investigate population structures, or reveal relatedness and parenthood. Here we analysed a set of 17 microsatellite markers commonly used for such analyses in the wild boar (*Sus scrofa*). Specifically, we considered genotypes of 123 females and 24 males originating from four different locations (enclosures) as well as their offspring from one year (n=110).

We ranked the microsatellite markers according to various criteria of suitability such as number of alleles, polymorphic information content (PIC), deviations from expected heterozygosity, likelihood of Null alleles, probability of identity (*P_ID*), and tested the performance of different microsatellite subsets resulting from our ranking (best 5, 9, 12 and all 17 loci, respectively) for a parenthood analysis and for assignment to their population origin. In addition, we used a Bayesian Structure analysis to identify genetic clusters in our data set and levels of individual admixture.

Individual identification was already reliable (*P_ID_sib* < 0.01, calculated by the GIMLET program) with the five best microsatellites in our data set. However, accurate parenthood and population assignment was only reached with the full set of 17 markers. Therefore, we conclude that a minimum set of microsatellites chosen according to the *P_ID* criterion does not provide enough statistical power for correct parenthood estimates or population assignment, albeit it would allow for individual identification and reduce laboratory costs.
PP-EB-04

Species Delimitation of Icelandic Decapods (Crustacea) using DNA barcodes and Automatic Barcode Gap Detection (ABGD)

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Species delimitation based on DNA barcode data is a field of many different approaches. Phylogenetic methods and statistical estimation of species number are used in a variety of analytical methods which have been tested in multiple studies on real and simulated data. We tested the applicability of one of those methods, the Automatic Barcode Gap Detector (ABGD), on barcode data of undetermined decapod specimens (Crustacea) which were collected during the “Icelandic marine Animals: Genetics and Ecology” (Ice AGE) expeditions. We show an efficient workflow from sequence data to primary species hypotheses (PSHs) using automatic analysis of the dataset including the Basic Local Alignment Search Tool (BLAST) and ABGD. Due to the fact that successful species delimitation depends on multiple prior assumptions the reliability of PSHs has to be tested. We developed automatic methods to analyse and visualize the results of our analysis and discuss the advantages and disadvantages of our approach.

PP-EB-05

Evolution in soil – phylogeography of soil-living arthropods reveals ancient radiations and few extinctions

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Phylogeographic studies on the European fauna and flora demonstrated that the Quarternary ice ages strongly affected species’ distribution ranges and genetic diversity. However, these studies predominantly investigated above-ground and aquatic organisms, leaving the question open, if the soil system was affected in a similar way. Soil-living organisms are small, with limited active dispersal ability, parthenogenetic reproduction is widespread and many above-ground organisms overwinter below-ground due to buffering of abiotic fluctuations by the soil. Therefore, patterns of genetic structure, recolonization and refuge areas of soil-living arthropods likely differ from those of above-ground animals. I will present a European wide phylogeographic study focusing on the most common soil-living soil arthropods i.e., oribatid mites (Acari, Oribatida) and springtails (Hexapoda, Collembola). General patterns of genetic variance, population structure and distribution ranges strongly differ from most above-ground species, indicating that evolutionary processes differ from the above-ground systems and that above-ground patterns cannot easily be transferred to the below-ground system. Interestingly, oribatid mites and springtails show consistent differences in genetic structure across Europe despite substantial similarities in their ecology and distribution ranges. As the poor man’s tropical rain forest, the soil system provides an underestimated playground to investigate eminent evolutionary questions.
PP-EB-06

An 18S-rRNA Variability Map for Rotifers

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18S ribosomal RNA represents one of the most widely used molecular markers for molecular phylogenetics, often at higher taxonomic levels. However, its utility extends beyond its sequence data (primary structure) to also encompass its specific, three-dimensional structure (secondary structure) comprising both conserved (stems of paired nucleotides) and hypervariable regions (loops and bugles of unpaired nucleotides). In this study, we present an estimate of the secondary structure of the 18S rRNA molecule in rotifers together with a variability map of the individual nucleotide positions based on sequence data from over 75 species distributed throughout the group and representing all major lineages (i.e., Seisonidea, Monogononta, and Bdelloidea). Our map not only provides an overview of the distribution of conserved and hypervariable regions for rotiferan 18S rRNA (especially in comparison to other major metazoan clades), but, together with our estimate of the secondary structure of the molecule, will help to design primers, to optimize alignments, or to improve phylogenetic analyses by enabling the use of doublet (or analogous) models of sequence evolution. In addition, we examine the potential for the 18S rRNA variability map to generate new, nDNA-based barcodes for rotifers.

PP-EB-07

Drosophila melanogaster’s fast responses to filamentous fungi

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As the frequently used common name “fruit fly” implies, Drosophila melanogaster makes use of fruits as breeding substrates. However, antagonistic microorganisms, such as filamentous fungi, often simultaneously occupy this type of substrate. Filamentous fungi can alter their environment by excreting substances, which hold detrimental functions to other organisms. The so-called chemical defence reduces competition with other microorganisms and fitness losses due to insect grazing, and so plays a major role in insect-fungus interaction.

We are investigating the transcriptional and evolutionary changes of D. melanogaster larvae to fungal infested substrate using whole transcriptome approaches. To disentangle the response of insect larvae towards a filamentous fungus and its toxic secondary metabolites (SMs) we have confronted D. melanogaster larvae with a wild type strain of Aspergillus nidulans (capable of synthesising SMs), the mutant strain DlaeA (impaired in SM synthesis), and sterigmatocystin as a representative of a mycotoxin produced by A. nidulans.

D. melanogaster larvae show a fast transcriptional reaction to fungal colonies that results in part in massive gene expression changes, which are specific to confrontation with the wild type fungus. On the other hand, the larvae’s response to the mycotoxin comprises a smaller set of genes and occurs at a later time.
PP-EB-08

The effects of microbiota on development and resistance in *Tribolium castaneum*

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The relevance of microbial communities inhabiting different animal species is increasingly being studied in a broad spectrum, ranging from sponges to primates. Although the red flour beetle *Tribolium castaneum* represents a well-established experimental model organism for studying questions in ecology, evolution and development, the relevance of its microbial communities is still unknown. Using a newly established protocol for microbe eradication and raising germ-free beetles, we found that individual, sterile *T. castaneum* larvae show a slower growth rate in comparison to the ones harboring commensal bacteria. Moreover, we demonstrate that upon an oral infection with the natural entomopathogen *Bacillus thuringiensis tenebrionis*, the survival of germ-free larvae is decreased in comparison to control animals, demonstrating thus a linkage between the commensal microbiota and the pathogen resistance of host animals. Therefore, this study represents a contribution to the accumulating evidence showing the importance of the microbiota in insects.

PP-EB-10

The virtual Lover - Do real fish interact with 3-D fish in questions of mate-choice?

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The fundamental question when studying mate-choice in animals is that of the relevance of information provided by certain behaviors or visual traits. What exactly triggers the choice of a female fish for a certain male and vice versa? To investigate this question we will use a virtual fish model presented on a screen that will interact with live fish in real-time during mate-choice experiments. The implementation of a new method should be followed by its evaluation.

Here, we present the first steps in designing a virtual photorealistic 3-D fish model of a sailfin molly (*Poecilia latipinna*). Data on perceptibility and acceptance of sailfin mollies towards this first prototype 3-D fish is shown. To pick up known constraints when using computer animations in behavioral experiments, we currently test the response of real fish to virtual fish. In binary choice situations, virtual fish versus real fish and video fish are presented. Additionally, we confront the use of LCD monitors with that of CRT monitors to evaluate possible technical limitations when presenting the virtual fish.

In the future, we will conduct experiments in which the virtual fish will also be able to interact with live fish. A new state of the art 3-D tracking system could already be established and will be assisting in the acquisition of behavioral data. The tracking system provides real-time data and was specially designed for the use with aquaria, considering refraction, mirror-images and shadows.
Not so desperate after all: does future life expectancy influence female mate choice?

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Bracon brevicornis (Wesmael) (Hymenoptera: Braconidae) is an arrhenotokous parasitoid wasp. Females mate once in their life and can actively reject male mating attempts. Based on the assumptions that (i) females with lower future life expectancy will invest more in current reproduction (terminal investment hypothesis) and (ii) females prefer symmetrical males over asymmetrical ones as mates, this study addresses the question of whether a female’s mate acceptance depends on her future life expectancy.

MHC - natural variation and mate choice in wild mice

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MHC genes exhibit an extremely high degree of polymorphism and heterozygosity. Despite decades of intense empirical and theoretical research, the evolutionary mechanisms of the origin and the long preservation of allelic diversity are not fully understood. MHC diversity has so far not been directly assessed in wild mouse populations, only for inbred strains derived from such samples. We aimed to identify the degree of natural polymorphism in different populations. Nevertheless, a clear picture of MHC influence on mate choice in natural populations of house mice cannot be drawn yet. This is partly due to the fact that the majority of the studies were carried out using lab inbred strains missing a natural genetic background. In a previous study we analyzed mate choice between two wild caught populations of *M. m. domesticus*, from France (F) and Germany (G). Individuals of both populations were kept in a semi-natural environment and had free choice of mating partners. F1 offspring showed a specific assortative pattern, i.e. a preference for paternally matching mates. This study raised the possibility of paternal genomic imprinting and/or early learning driving the decision for a certain mating partner. The current study aims to assess the degree of natural variation at MHC in several wild mouse populations, as well as to elucidate MHC-driven mate choice behavior/social preference in house mice in the wild and under controlled - cage conditions, respectively.
PP-EB-13

Do female zebra finches generalise a socially learned mate preference for novel male phenotypes?

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Social information influences mate preferences and can even lead to a preference for novel phenotypes. Previous experiments have shown that zebra finch females copy the mate choice of other females and prefer a novel male phenotype when they have seen another female interacting with her adorned mate. Here, we tested whether zebra finch females (Taeniopygia guttata castanotis) would generalise a socially learned preference for a novel male phenotype (adorned with a feather) to another novel male phenotype (adorned with another feather). Females could choose between a red or blue adorned and an unadorned male in a first mate-choice test. Afterwards females could observe a single unadorned male and an adorned male (other color) with his unadorned mate for two hours. Following this observation period, females could choose between two new males, one red or blue adorned (same as in first test) and one unadorned, in a second mate-choice test. In two controls, adorned males wore either a blue feather during both mate-choice tests, and a single unadorned and a single blue adorned male were presented in the observation period, or adorned males always wore a red feather and two single males were presented in the observation period. Females never preferred one over the other male during the first and second mate-choice test and there were no changes between the mate-choice tests. We conclude that females do not generalise a preference between two different novel male phenotypes. Therefore, the social information gathered about a new sexual trait was not generalised to another similar trait.

PP-EB-14

Female mating preferences and male coercion in Crested Macaques

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The exaggerated swelling hypothesis predicts that female primates in multimale groups use their sex skin swellings to exert their fertility dependent mating preferences and in this way fulfill their need to confuse paternity as a counterstrategy against male infanticide, while at the same time concentrating paternity in preferred males. According to this theory, females should attempt to mate with many males outside and with the preferred male during the fertile period of the ovarian cycle. In how far female mating preferences conflict with male interests, and to which degree they are limited and manipulated by male coercion, however, still remains to be investigated. The aim of our study was to determine 1. female mating preferences during and outside periods of female fertility; 2. the degree of male coercion and whether coercion was linked to apparent female preference in crested macaques (Macaca nigra), a species in which females show exaggerated sex skin swellings. In addition to the predictions by the exaggerated swelling hypothesis, we predicted that the number of copulations with a certain male would increase with increasing aggression by and female preference for this male. Data were collected on two habituated wild groups in the Tangkoko Reserve, Indonesia from July 2006 to July 2007. Behavioural data were collected during all day focal animal sampling of 19 females over 31 cycles. The timing of ovulation was determined using faecal progesterone. Our results will further our understanding of the co-evolutionary processes deriving from intersexual conflict of reproduction and their influence on male reproductive success.
**PP-EB-15**

**Dynamic resource allocation in pre- and postcopulatory sexually selected traits in three-spined sticklebacks (Gasterosteus aculeatus)**

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Male reproductive success in polyandrous mating systems depends both on (precopulatory) mate-acquisition traits and (postcopulatory) sperm competitive abilities. The investment in these traits is generally mediated by resource availability as an increased allocation in one function often results in a decreased resource availability for alternative functions. During the breeding season reproductively active three-spined stickleback males (Gasterosteus aculeatus) develop a conspicuous carotenoid-based breeding coloration in the cheek region, which is a target of female choice. Moreover, the risk of sperm competition is known to be high in this species. Thus, in sticklebacks both episodes of sexual selection are known to be of importance for reproductive success. In the present study, we used laboratory bred stickleback males to examine how resource availability in terms of experimentally manipulated diet quality (carotenoid content) and quantity affects the expression of pre- and postcopulatory sexually selected traits. Individuals were raised under standardized conditions and underwent three different feeding treatments: (1) high-quality/high-quantity, (2) high-quantity and (3) low-quantity. Interestingly, food-restricted males developed on average a more intense breeding coloration and faster sperm compared to their well-fed brothers. Thus, resource-limited males allocated relatively more in both pre- and postcopulatory traits, possibly to maximize present reproductive success at the expense of future reproduction. In accordance with sperm competition theory a trade-off between the investment in pre- and postcopulatory traits was observed within the food-restricted group. In contrast, well-fed males were able to invest equally in both traits resulting in a positive relationship as predicted by the phenotype-linked fertility hypothesis.

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**PP-EB-16**

**Age-dependent sperm allocation in virgin copulations triggered by male age not mating history in the lesser wax moth Achroia grisella**

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Fertilization opportunities normally decrease with male age and own previous research has demonstrated that male wax moths Achroia grisella - a short-lived, aphagous, lekking moth - strategically increase sperm allocation in virgin copulations with age. However, it remained unclear whether transferring a higher proportion of available sperm with older ages is triggered by male age per se or rather by the time elapsed with no opportunity to mate (mating history), which may be used by males to assess the density of reproductive females or their own attractiveness. To identify the selection pressures acting on strategic sperm allocation in this system, we staged experimental matings between virgin females and eight-day-old non-virgin males which differed in mating history (age of their virgin mating one versus four days) and analysed copulation behaviour and sperm expenditure. We present clear evidence that age-dependent strategic sperm allocation is triggered by male age per se and discuss its implications for the significance of post-copulatory sexual selection in this system.
PP-EB-18

Conditional strategies and their implications for the joint evolution of sexual selection and paternal care

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Traditionally, sexual selection theory has focused on the evolution of female preferences in organisms where males do not provide ‘direct benefits’ to their mates. However, in many taxa (e.g. the majority of bird species; some fish, insect and mammal species) males contribute substantially to parental care, an important but costly behavioural trait enhancing reproductive success. The question arises whether ornamentation can evolve in such species if the allocation of resources to an ornament compromises the amount and quality of care provided. We addressed this question by an evolutionary modelling approach involving individual-based simulations. In the models, males had to decide on the fraction of their resources to be allocated to paternal care (thereby enhancing the survival probability of their offspring) and to ornamentation (thereby enhancing their success on the mating market). If allocation decisions had to be made before a male’s resource level was known, neither male ornamentation nor female preferences did evolve. However, evolution of preferences and ornamentation did occur when males could make their allocation decision dependent on the amount or quality of their resources. In that case, the evolved conditional allocation strategies had the property that the degree of male ornamentation is a reliable signal of a male’s paternal quality. Our study demonstrates that sexual selection models incorporating conditional parental strategies will often lead to very different conclusions than models neglecting such flexibility.

PP-EB-19

Parental care mitigates detrimental effects of maternal presence under food limitation in an insect with facultative family life

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Family life is usually associated with different forms of care that promote offspring fitness, but also entail costs for parents. Harsh environmental conditions have recently been shown to exacerbate these costs in species with non-obligatory family life, ultimately rendering parental presence detrimental for offspring fitness. However, whether and how parents and/or offspring mitigate this detrimental effect remains unexplored. Here, we addressed these questions in the European earwig (*Forficula auricularia*), an insect with non-obligatory family life in which food restriction renders maternal presence costly in terms of offspring survival. We asked whether females can mitigate the negative effect of their presence through offspring provisioning and whether this behavior is costly in terms of future reproduction. Because offspring in this species are known to transfer food to their siblings, we also investigated whether this food transfer influences the negative effect of maternal presence. We assessed nymphal survival and female reproductive investment in current versus future reproduction, as well as maternal food provisioning and food sharing among offspring in 100 clutches that had been split into two equally sized groups and raised either with or without mother under food restriction. Our preliminary results indicate that the detrimental effect of maternal presence on offspring survival decreases with increasing maternal food provisioning, but is not affected by food transfer among offspring. This suggests that females, but not offspring, can mitigate the costs of female presence and highlights the importance of parental care under harsh environmental conditions.
PP-EB-20

Clutch size decisions and size-fitness relationship in the parasitic wasp, *Bracon brevicornis*

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One of the trade-offs parasitoid wasps have to deal with is the one between offspring number and offspring quality: Once a host has been encountered a female decides how many eggs to allocate, as the resource is limited and sibling competition occurs in gregarious parasitoids. Therefore, a female is expected to vary clutch size as a response to resource availability (here measured as host weight), to maximize the fitness return. This is often called the Lack clutch size. The influence of clutch size on body size of emerging adults has been studied, and results show that larger clutches often give rise to smaller adults. This study examines the relationship of host size and clutch size in the ectoparasitoid wasp *Bracon brevicornis*, as well as the relationship between the resulting daughters’ body sizes and their fitness. Results provide support for optimal clutch size theory, and larger daughters were found to have higher egg production, lay bigger clutches, and produce better quality offspring themselves. However, it was not possible to relate daughter body size to longevity, another important component of fitness.

PP-EB-21

The evolution of major regulators of sex determination in social insects

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The common principle of sex determination shared by all hymenopteran species is haplodiploidy. Its molecular basis is best understood in the honeybee *Apis mellifera*, harbouring a single multiallelic gene, complementary sex determiner (*csd*) which acts by heterozygosity as primary signal for female development. The downstream target of *csd*, its paralogous copy *feminizer* (*fem*), is sex specifically spliced mediated by *transformer2* (*tra2*). In the closely related buff-tailed bumblebee (*Bombus terrestris*) the mechanism of sex determination is barely known. We focus on the molecular evolution of these regulators within the sex determination pathway using newly sequenced social insect genomes. We aim to characterize genes involved in sex determination in *Bombus* by different assays of protein-protein and protein-RNA interaction. Our comparative evolutionary and genetic analyses show that the parologue of *fem* in *Bombus*, *fem1*, has no allelic variability which is in strong contrast to *Apis csd*. The presumably absence of *fem* duplicates in other bee species supports the hypothesis of independent *fem* duplication events among phylogenetic lineages. *Fem1* might has developed a novel function in *Bombus* like *csd* in *Apis*. We assume two different hypotheses: 1.) a so far unknown multiallelic gene acts as pre-signal for *fem1* and 2.) *fem1* is not involved in sex determination and another gene mediates sex specific splicing we observed for *fem*. The *tra2* gene, a protein-RNA-binding cofactor seems differently evolved in *Apis* and non-*Apis* bee lineages. We use *in vitro* expressed *tra2* protein to detect potential interaction with *fem*, *fem1* and to so far unknown genes associated to the pathway to broaden our understanding of the sex determination mechanism in bees.
**PP-EB-21**

Temporal stability and strength of social bonds in dispersing male assamese macaques

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In multi-male multi-female primate groups, the strength and stability of social bonds has been shown to affect individual’s long-term fitness (e.g. longevity, siring success and offspring survival). Yet studies investigating the fitness benefits of close social relationships and the underlying mechanisms mainly focused on the philopatric sex. These studies described long lasting, strong and equitable relationships of certain dyads and highlighted how their stability and strength was shaped by kinship, rank and age. In contrast, only one study so far, on male Assamese macaque (*Macaca assamensis*), demonstrated the fitness benefit of social bonds in the dispersing sex. Here we aim at investigating whether characteristics of social bonds, such as stability and strength, resemble those found in philopatric female baboons and philopatric male chimpanzees. We use focal animal data of social interactions collected over 8 years on two wild groups of Assamese macaques at Phu Khieo Wildlife Sanctuary, Thailand. We quantified the strength of social relationships between adult males and the consistency of the top social partner choices between years. We found that male-male social relationships are differentiated in strength from weak to very strong bonds and that these differences in strength remain stable over several years. This fosters our understanding of social dynamics in polygynous primate groups and of the proximate mechanisms underlying the formation and maintenance of social bonds.

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**PP-M-01**

Immunolocalisation of pigment-dispersing hormone in the embryonic nervous system of an onychophoran: A comparison to arthropods

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Pigment-dispersing hormone (PDH) is involved in modulating eye pigment migration in crustaceans and is part of the circadian clock network in insects. Studies on PDH-immunoreactivity (PDH-ir) in the adult and embryonic nervous systems of insects and crustaceans revealed a similar arrangement of neurons that label for this peptide. Since comparative data are missing from one of the closest arthropod relatives - the Onychophora - we examined the ontogeny of PDH-ir in the central nervous system of the velvet worm *Euperipatoides rowelli* Reid, 1996 using the 3B3 antiserum against PDH. Our data show that the initial PDH-ir somata emerge at the anterior-most (=protocerebral) commissure in the developing onychophoran head. During further development, a complex system of additional PDH-ir somata and neurites is formed within the brain and the ventral nervous system. In contrast to insects and crustaceans, these somata do not exhibit bilateral symmetry or segmentally repeated arrangement in brain neuromeres and ventral nerve cords, which we consider as a plesiomorphic condition. Moreover, in contrast to crustaceans, the PDH-ir somata of onychophorans are located in the developing ventral nerve cords and project their neurites anteriorly towards the protocerebrum. Furthermore, the PDH-ir somata of insects and crustaceans are mainly associated with the optic neuropils, whereas no PDH-ir somata and only a few neurites occur in the optic neuropile of *E. rowelli*. These findings suggest that PDH is unlikely to modulate migration of eye pigment and plays only a marginal role as a circadian pacemaker in onychophorans.

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**PP-M-02**

**Pigment-dispersing hormone immunoreactivity in the brain of *Lithobius forficatus* (Myriapoda, Chilopoda)**

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Pigment-dispersing hormones (PDHs) are octadecapeptides, which play a regulatory role in modulating pigment migrations in the visual system of decapod crustaceans. Furthermore, they probably function as a pacemaker in the circadian clock network in hexapods and crustaceans. Thus, in Tetraconata PDH-immunoreactive neurons are integrated in neuronal networks and likely play a role in the endogenous clock. Previous investigations in the adult and embryonic nervous systems of hexapods and crustaceans showed that the expression patterns of PDH seem to be quite similar. To date, studies on PDH in representatives of Myriapoda are absent and it is not clear whether PDH-immunoreactivity (PDH-ir) is likewise present in the protocerebrum of mandibulate arthropods. Therefore, we investigated the PDH-ir in the chilopod *Lithobius forficatus*. Similar to Tetraconata, PDH-ir structures are mainly located in the median and lateral protocerebrum and comprise two dominant commissures. In addition, PDH-ir is also present within the tritocerebrum and the subesophageal ganglia. Furthermore, PDH-ir somata are located near the second optic neuropil (commonly termed medulla) which are in a corresponding position to those found in hexapods and crustaceans.

**PP-M-03**

**Variation of leading edge serrations of owls (Strigiformes)**

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Owls (Strigiformes) are mostly nocturnal birds that are known for their silent flight. The silent flight is enabled by several morphological adaptations. Adaptations to a silent flight are a low wing loading and specialized feather structures. The feather specializations are a velvet-like dorsal surface, a fringed trailing edge and serrations at the leading edge. The leading edge serrations prevent disadvantageous aerodynamic effects. Additionally it is assumed that the serrated leading edge reduces noisy air flow structures at the wing. The shape of the leading edge serrations has been described for several owl species. However no systematic studies regarding the shape and distribution of the serrations at owl wings has been done yet. In this study we did a morphometric comparison of the shape and distribution of leading edge serrations at the wings of different owl species. We investigated species of different size to obtain insight in the properties of the leading edge serrations that may be important for the air flow alteration. Furthermore we compared the wings of nocturnal species with such of diurnal species. By this comparison we wanted to evaluate the importance of the leading edge serrations on noise reduction. Preliminary data show that the serrated leading edge extends over the distal part of a wing. For owl species with slotted wings serrations are further present on detached feather tips. Results so far indicate a relation of species size/activity to serration size but no relation to the distance between adjacent serrations.
PP-M-04

Attachment ability of *Drosophila melanogaster* mutants with defective pulvilli on various substrate roughness

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With their hairy adhesive pads, wild-type flies *Drosophila melanogaster* are able to walk on smooth vertical surfaces. The adhesion-defective *Su(z)2*-mutant allele *Su(z)2handGFP1.1* lacks this ability. To reveal the difference in the attachment ability between wildtype and homozygous mutant animals on different substrate roughnesses, the maximum climbing angle on various substrate casts was tested. Additionally, we examined morphology of the adhesive pads by means of scanning electron microscopy and studied the presence of the fluid secretion in spatula contacts using high-speed video recording in combination with reflective interference contrast microscopy. Our results show that the mutant’s ability to climb on all tested substrates was significantly decreased in comparison to the wildtype, especially on smooth surfaces. Morphological analyses of the mutant showed a general shortening and higher inflexibility of the adhesive pad basis and a decrease in the number of adhesive setae. High-speed video analyses revealed that mutants do not produce any wet footprints on glass due to the inability to touch the substrate with their adhesive pads. The contact with the substrate is only achieved by claws. This explains the mutant’s comparably higher attachment on rough surfaces.

PP-M-05

Origins of the ptychoid defense mechanism in oribatid mites (Acari) - a morphological view on the respective sister groups

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Oribatid mites represent an important part of soil decomposer systems. Their— for chelicerates unusual— feeding mode (particle feeding) and use of relatively low quality food have been considered constraints resulting in comparatively slow movement, low reproductive potential and prolonged generation time. As possible resource for soil predators, oribatid mites evolved various defensive strategies to achieve the long adult life necessary for reproduction. The most complex mechanical defensive mechanism is ptychoidy, in which the animals can effectively retract their legs and gnathosoma into the idiosoma and encapsulate themselves by deflecting the prodorsum. This is enabled through a system of exoskeletal and muscular elements. Ptychoidy probably evolved independently in three groups: one time each in the Ptyctima, the Mesoplophoridae and the Protoplophoridae. The independent evolution of such a complex morphological feature suggests some form of preadaptation in the respective groups and the ground plan of the oribatid ancestor possibly featured characters that facilitate its evolution.

To investigate and compare the morphology in all three groups and their alleged respective sister groups we used synchrotron X-ray microtomography. We show that all sister groups (genus *Collohmannia* and *Epilohmannia*, *Eniochthonius*, and *Sphaerochthonius*) exhibit characteristic features associated with the ptychoid defense mechanism of the Ptyctima, Mesoplophoridae, and Protoplophoridae, respectively. E.g., the muscles associated with the build-up of pressure in the Ptyctima show a striking similarity with homologous muscles in the non-ptychoid *Collohmannia* and *Epilohmannia* species.
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The Corethrellidae (“frog-biting-midges”) is a small group among the lower Diptera. The family contains approximately 110 species. Their predaceous larvae feed mainly on other insect larvae e.g. mosquito larvae. They capture prey with their antennae, a mechanism which also can be found in the closely related Chaoboridae. This unusual feature and the lack of morphological data of internal structures induced us to investigate the cephalic structures of the larvae of *Corethrella appendiculata* (Grabham 1906). The closely adjacent antennae are one segmented and insert on the anterior margin in a horizontal position in specific pockets of the head capsule. A strongly developed antennal muscle causes the abduction of the antennae leading to catch prey. In larvae of Chaoboridae the antennae are also located on the anterior margin of the head, but in a vertical position. In contrast to Corethrellidae the abduction is caused indirectly by increasing the hemolymph-pressure. The adduction is caused by the antennal muscle. Because of this unique mechanism a sistergroup relationship of Corethrellidae and Chaoboridae is confirmed.

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A bird feather consists of a shaft, a rachis and inner and outer vanes composed of barbs. The material properties of the barbs influence the aeroelastic and aeroacoustic behavior of a bird wing. We investigated the material behaviour of feather barbs of barn owls (*Tyto furcata pratincola*) and pigeons (*Columba livia*) in relation to the silent flight of owls. Two series of tensile tests were carried out. In the first series, barbs were taken from three positions along a barn owl feather and their tensile behavior was measured in the middle of their lengthwise extent. In the second series three positions along the lengthwise extent of barbs from barn owls and pigeons were tested. Furthermore a finite element simulation of a barb was developed and evaluated by comparison with barb bending tests. According to this comparison the simulation systematically underestimated the forces in bending, rendering between 24% and 52% of the values. In the tensile tests the barbs exhibited a linear increase of stress with strain between 0.8% and 5% elongation. Correspondingly the Young’s modulus, a measure of elastic material stiffness, was determined as the slope in this range. The first series revealed that along a barn owl feather, the Young’s moduli are particularly high at the inner vane at 10% rachis length. Along barbs from barn owls as well as from pigeons, a gradient of Young’s modulus was found, with higher values towards the tips of barbs. The data suggests that the gradients observed did not evolve in conjunction with the silent flight of owls. Higher Young’s moduli in the barbs tips can be aerodynamically beneficial as they provide stiffness in spite of a tapered form.
Morphology

**PP-M-08**

3D geometric morphometric analysis of the breast-shoulder apparatus: implications for the locomotor kinematics of anole (Squamata: Dactyloidae) ecomorphs on Caribbean islands.

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The breast-shoulder apparatus (BSA) of lizards (consisting of the ribcage, presternum, interclavicle, paired clavicles, and paired scapulocoracoids) is a structurally and kinematically complex entity. The variability of this complex has seldom been the focus of functional explanation, possibly because the BSA has been difficult to analyse and compare as a composite contrivance. Here we apply geometric morphometric techniques to the analysis of the BSA *in situ*, in an attempt to more fully understand its configuration in relation to differential use in locomotion. Our approach centers upon anoline lizards, a cluster that has been extensively studied from an ecomorphological perspective. The species selected for study represent distantly related forms of both the trunk-crown and trunk-ground ecomorphs sourced from Hispaniola, Jamaica, and Puerto Rico. We hypothesized that species will exhibit variation in the configuration of the BSA that is chiefly explained by its ecomorph assignment. Our findings indicate that the three-dimensional configuration of the shoulder girdle of island anoles is directly associated with specializations related to habitat occupancy (ecomorphology). The members of each ecomorph studied share morphological similarities that have been derived independently, rather than being shared through phylogenetic history. This indicates that the form and function of the BSA is subject to convergent evolution within the anoles of the Greater Antillean islands, in a similar manner to the external morphological traits that have been employed to characterize the various ecomorphs.

**PP-M-09**

Chemical communication in Argiope bruennichi (Araneae) - SEM-analysis of chemosensilla of males

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Olfaction is considered the prime channel of communication in spiders, irrespective of whether they are cursorial or web-building. However, we know surprisingly little about which chemical signals or cues are involved in olfaction and only a single pheromone responsible for male attraction to virgin females is known to date. Even less is known about the receptors involved: s-shaped and blunt tipped sensilla with a subterminal pore opening are considered to be responsible for detecting volatile substances in spiders. So far, electrophysiological analysis demonstrated the perception of contact pheromones with these sensilla and it is generally assumed that the same structures are responsible for long distance olfaction.

Many facets of the mating system of the orb-weaving spider *Argiope bruennichi* are known including the fact that virgin females signal their receptivity to males by means of a volatile pheromone that is not emitted by subadult and mated females. Since the structure of the pheromone (methyl-methlycitrate) has been clarified, the pheromone can be synthesized for electrophysiological analysis. However, as a first step the structure and distribution of the supposed chemosensilla needs to be investigated. To this aim, we scrutinized legs and palps of *A. bruennichi* males for tip-pore sensilla by means of Scanning Electron Microscopy, described their structure and constructed a distribution map for each extremity. The results show that *A. bruennichi* males possess chemosensilla on all legs and the palps and that these structures are particularly common on tarsi and metatarsi, decrease in number on tibiae and patellae and are absent on coxae and trochanters. On the male pedipalp, chemosensilla only occur on the cymbium. Further electrophysiological studies will test if males are able to perceive the female pheromone with these sensillae and perform mate choice accordingly.
Morphology

PP-M-10

Pattern and density distribution of photoreceptors in the zebrafish retina

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The retina of teleosts is characterized by a species-specific mosaic pattern of cone photoreceptors. For further research concerning the geometry of synaptic connectomics, we examined the photoreceptor arrangement and density distribution in the zebrafish retina to localize the region with the highest cone density (“area”).

A zebrafish retina was fixed in glutaraldehyde, dissected into 16 fragments, and embedded in epoxy resin. Each retina fragment was serially sectioned (1.5 µm) from the pigment epithelium to the outer limiting membrane, stained with Richardson’s reagent and photographed under a light microscope (40x objective). Using Amira® Software, these digital image stacks were aligned and segmented for surface rendering of the cones. Finally the cone density was determined in Photoshop®.

For a parallel approach a retina was fixed in formaldehyde, stained with DAPI and the 3D pattern of cell nuclei was imaged using a 2-photon laser scanning microscope for subsequent metric analysis.

The investigated retinae of the zebrafisch, Danio rerio, show the expected mosaic pattern, which consists of alternating rows of (unequal) double cones and (long and short) single cones. The cone density though varies within the retina, possessing the highest density in the ventral region.

The four cone types in the zebrafish retina, distinguishable morphologically and by their visual spectrum, enable sharp tetrachromatic color vision. The density distribution indicates that the region of greatest visual acuity should be within the upper field of vision. A 3D-EM connectomics study will be carried out in the “area”-region, in which the receptive fields of second order neurons are expected to be comparatively small.

PP-M-11

Sister where are you? The phylogenetic position of the genus Attaphila Wheeler, 1900 (Dictyoptera, Blattodea)

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Attaphila Wheeler, 1900 are small wingless cockroaches that live in the nests of leafcutting ants of the genera Atta and Acromyrmex. Six species have been described from the Neotropical Region and the Southern United States. Attaphila fungicola has two hosts, while all other species are known from one host ant each; the cockroaches live in the tunnels of the fungus gardens. Attaphila are ovoid in shape, and covered with yellowish hairs. Tegmina and wings are vestigial in males, and completely absent in females. The legs of Attaphila have a large ariolum, which enables both adults and nymphs to attach themselves to their hosts during swarming. This mechanism allows the species to be widely distributed. The terminal maxillary palpomeres have a unique structure that is used to detect and follow ant trail pheromones. The phylogenetic relationships of Attaphila to other cockroaches are still unknown, as is the morphology of their genitalia. We present our first results from a detailed study of several character systems. The focus is on the morphology of the proventriculus, the male and female genitalia, and the leg spination. The morphology of the male genitalia suggests a placement of Attaphila in the Blberoidea (including the paraphyletic Blattellidae and the Blaberidae), which apart from the Cryptocercidae-Isoptera clade is the most species-rich lineage of Blattodea.
PP-M-12

Fast and sticky - tongue adhesion in the salamander *Eurycea guttolineata*

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It has been observed that the ballistic tongue projection mechanism in plethodontid salamanders (Caudata: Plethodontidae) is one of the fastest movements performed by vertebrates. Peak accelerations of the salamander tongue can be up to 1992 ms⁻². The high velocities of tongue projection in salamanders are supposed to put special demands on the adhesive tongue pads in these salamanders since the time frames for contact formation are extremely short. Among plethodontids, ballistic tongue projection evolved at least three times independently. One of the three lineages with ballistic tongues are salamanders of the genus *Eurycea*. Here we studied the functional morphology of tongue projection in *Eurycea guttolineata* to clarify the feeding kinematics and to measure tongue adhesion forces. For this we combined modern imaging techniques such as high-speed video, SEM, and micro-CT, enzyme clearing and staining, and force measurements. Micro-CT imaging revealed the three-dimensional structure of the muscles acting on the tongue, i.e. the m.subarcualis rectus and the m.rectus cervicis. Highspeed-Video-Imaging suggests that the time to establish a contact with a prey item is only approximately 4ms, which is much shorter than e.g. in frogs with projectile adhesive tongues. Preliminary data of the force-measurement against a glass surface shows that the forces effectively might be in range of the body weight of the corresponding animal which is in the range of 50 to 60mN which will clearly outweigh any potential prey item for these salamanders. Our results present the first quantification of tongue adhesion in salamanders.

PP-M-13

Next generation histology in comparative morphology

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Modern microscopical methods like µCT, FIB, and cLSM have almost replaced traditional histology in the comparative study of animal anatomy. The main advantage of these modern methods is the provision of volume data that represent anatomical structures in their original arrangement and thus enable automated image processing for anatomical 3D reconstruction.

Histology on the other hand provides a higher resolution at cellular level and profoundly eases unambiguous interpretation of the data due to differential, tissue specific stains. Yet, the use of serial section histology for 3D reconstruction underlay three drawbacks: 1) Image data must be acquired section by section and transformed into an aligned image stack. 2) Structure labeling in terms of segmentation of images cannot be automated. 3) High resolution image stacks comprise several gigabytes of data and thus are difficult to handle by software for processing and publication. The latter impedes the sharing of original data sets and forces researchers to rely on morphological descriptions and diagnoses provided by others. Here we present a standardized workflow for serial section histology to circumvent these difficulties. In short, we use a semi-automated microscope to acquire high resolution image data. The images are collected into a stack and aligned semi-automatically. The subsequent conversion of the image data into small tiles allows manual segmentation at any level of resolution. The data are finally deposited in the database MorphDBase where they can be accessed freely by other researchers. A survey in brain morphology of diverse bilaterian taxa demonstrates that this workflow fastens serial section histology dramatically and results in comparable, openly accessible and well-documented research data.
Adapted scale morphology and sediment interaction in four European flatfish species (Pleuronectidae)

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Flatfishes (Pleuronectidae) bury themselves in the sediment for camouflage and protection against water currents. Whereas species-specific preferences for sediments of certain grain sizes were previously shown in the literature, the role of scales in interaction with sediment has not been investigated. We have studied the scale morphology and the critical sliding angle of sediments on the skin for different grain sizes in four European flatfish species: Limanda limanda, Platichthys flesus, Pleuronectes platessa, and Solea solea. The species had different scale types ranging from deep-embedded cycloid scales to ctenoid scales that are exposed to the sediment. The sliding angle of sediment was significantly different among the fish species and the different directions on the fish (craniad, caudad and laterad). Our results provide an evidence for the impact of scale morphology on the sediment sliding and give an insight about the ability of scales to keep sand on the fish surface. Since the critical sliding angle depends on grain size, our results suggest that the morphology of flat fish scales may be adapted to the specific grain size of the sediments of their preferred habitats.

The role of hydraulic pressure and pumping activity in body contraction and expansion cycles of Tethya wilhelma

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A high number of sponges are able to contract their body in a coordinated and regular manner. This phenomenon is frequently observed in Demosponges and has been demonstrated for Calcispongia, Homoscleromorpha and Hexactinellida as well. From the analysis of time lapse image sequences detailed information on the kinematic of such body contractions is available and it has been show that a contraction cycle can be divided into four distinct phases distinguished by the velocity and acceleration of the contraction and expansion phase. Pinacocytes have been identified as main effectors of the body contraction. However, only little is known about the antagonistic system. We have studied the role of hydraulic pressure and pumping activity in the aquiferous system of Tethya wilhelma during body contraction and expansion cycles. A combined analysis of body contraction kinematics and pumping activity was performed using a time lapse particle tracking velocimetry approach. We have studied the oscular contraction cycle in correlation to body contraction cycles using digital timelapse sequences. Our results displayed an onset of pumping activity shortly after the maximum body contraction has been reached. However, the oscule remained closed until the fourth phase of the body contraction is reached, which is characterized by a slower expansion velocity. We therefore propose a substantial role of hydraulic pressure generated in the aquiferous system as antagonist to contractile elements (pinacocytes) and effector in the expansion phase of body contractions in T. wilhelma.
PP-M-16

Living in oblivion: Histological study of the Grueneberg ganglion, a rarely regarded mammalian olfactory organ

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The Grueneberg ganglion, named after its discoverer Hans Grüneberg (1973), currently represents the least explored of the four olfactory organs known in mammals (the other three being the main olfactory epithelium, the vomeronasal organ and the sepal organ). It is localized within the dorsal tip of the nose, embedded into the connective tissue right below the (often keratinized) epithelium of the anterior nasal cavity. Hitherto only very few studies exist that cover the Grueneberg ganglion; the functional properties of this supposedly olfactory structure and in particular its distribution among mammalian taxa is largely unknown. As part of a larger comprehensive investigation we herein present the first description of the Grueneberg ganglion in Tupaia glis (common treeshrew, Scandentia) and Sorex araneus (common shrew, Soricomorpha). The collected data give detailed information on the topographic position as well as on the anatomical characteristics of the ganglion cells, which are mainly arranged in cluster-like structures. Based on high resolution microphotographs we document the histological morphology of single ganglion cells and their associated satellite cell sheaths.

The present results can be seen as a first stepping stone in an attempt to determine the general occurrence as well as the evolutionary origin of the Grueneberg ganglion within mammalian vertebrates.

PP-M-17

Olfactory projection neuron pathways in marine Isopoda and Remipedia

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The anatomy of the olfactory projection neuron pathway has been studied in several representatives of Malacostraca. Nevertheless, the morphology of olfactory projection neurons and second order olfactory neuropils are discussed rather controversially in Peracarida. Therefore, we investigated the olfactory projection neuron pathway of the marine isopods Saduria entomon and Idotea emarginata, using focal dye injections of the lipophilic tracer Dil into the olfactory neuropil. Both arms of the olfactory globular tract form a chiasm in the center of the brain and the olfactory projection neurons innervate the medulla terminalis and the hemiellipsoid body of the ipsi- and the contralateral hemisphere. Both protocerebral neuropils are innervated to a similar extent. This is reminiscent of the situation in the basal decapod taxon Dendrobranchiata. Thus, we propose that an innervation by the olfactory globular tract of both the medulla terminalis and the hemiellipsoid body is not only part of the decapod ground pattern, but also of the ground pattern of Caridoida. This proposal is based on the similar arrangement in Dendrobranchiata and Isopoda. The recent finding of hemiellipsoid bodies in certain Peracarida suggests a wider distribution of hemiellipsoid bodies in Malacostraca than previously thought. Chemoneuroanatomical data regarding cAMP signaling in the olfactory projection neuron pathway of Remipedia, a key taxon in the debate of tetraconate phylogeny, are considered in an evolutionary context.
PP-M-18

Morphology and systematic implications of the caruncle in the males of four species of Limnonectes (Anura: Dicroglossidae)

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The males of four species of the Asian frog genus Limnonectes (L. dabanus, L. gyldenstolpei, L. macrognathus, L. plicatellus) exhibit a remarkable ornamentation in form of a swollen, or cap-like structure (caruncle) on the top of their head. These caruncles vary in their appearance among species and neither their function nor actual systematic value is known. We compared their anatomy via dissections, morphometrics, radiography and histology, and also analysed both available mtDNA sequences as well as new data to place these species within the context of a larger phylogenetic hypothesis for Limnonectes. Despite the externally different morphology, the underlying histological structure is virtually identical. Beneath skin densely packed with mucous glands, lies a pad of connective tissue overlaying the parietal bone. The actual function of the caruncle, however, remains enigmatic. In addition to the presence of the caruncle, independent evidence from osteological characters and molecular data support the monophyly of a clade comprising of L. dabanus, L. gyldenstolpei, L. macrognathus and L. plicatellus. The caruncles therefore are interpreted as a robust autapomorphy for this clade and suggest that the subgenus Elachyglossa should be restricted to the four species in question.

PP-NB-01

From Hearing to singing: sensory to motor information processing in the grasshopper brain

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Grasshoppers, and among them especially the species Chorthippus biguttulus, have been used as a model system to study insect acoustic behaviors since 25 years. The behavior and various neurological mechanisms underlying song recognition and song production are well described. Numerous studies identified the set of Auditory neurons in the thorax and the way they process information. Projection of ascending neurons to the brain have been identified among various species. Since only few data exist about brain neurons involved in acoustic pattern recognition, the neuronal basis of the song is still poorly understood. Particularly there is no information about correlated or synchronized activity of larger sample of auditory neurons which might indicate representation of particular features of auditory signals in the different types of neurons.

This project aims at elucidating important steps of neuronal processing involved in the recognition of specific-specific acoustic communication signals and in the selection of appropriate acoustic responses. It will employ extracellular multi-unit recordings with tetrodes. Successful completion of project will increase our knowledge about neural mechanisms of the song pattern recognition and the coupling of higher sensory networks for song recognition with higher motoric areas select and coordinate appropriate responses.
PP-NB-02

Spider silk as guiding biomaterial for human neurons

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Due to its antimicrobial and wound-healing activity, spider silk has a long and rich history of use in folk medicine. Spider silk is a fibrous biomaterial of exceptional tensile strength which is non-immunogenic in the human body and completely biodegradable. Here, we explore the use of spider silk fibers as a guidance material for axonal regeneration after neural injury. We used a cell culture assay to test the compatibility of drag line spider silk from *Nephila* spiders with human neurons. These model neurons were differentiated by retinoic acid treatment from the Ntera2/D1 clone of a human teratocarcinoma cell line. We have previously demonstrated that Ntera2 neurons can form functional neural networks. To visualize cellular interactions between spider silk and the neurons in vitro, we developed a crossed silk fiber array. We describe for the first time that human neurons attach to silk scaffolds. Extending neurites can cover gaps among single silk fibers and elongate afterwards on the neighbouring fiber. Culturing human neurons on the silk arrays led to an increasing migration and adhesion of neuronal cell bodies to the spider silk fibers. Within three to four weeks, the clustered somata and extending neurites formed ganglion-like cell structures. Microscopic imaging of human neurons on the crossed spider silk fiber arrays in vitro will allow for modifications to maximize cell adhesion and axonal growth prior to translating these findings into neuroregenerative therapy.

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PP-NB-03

Comprehensive analysis of neuroglobin expression patterns and phenotypic analysis of a neuroglobin KO mouse by RNA-Seq

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Despite ~14 years of intense research, the function and even the expression sites of neuroglobin (Ngb) are still a matter of debate. In order to interpret the physiological function(s) of Ngb in relation to its gene expression pattern, we evaluated murine Ngb expression by extensive bioinformatic analysis using publicly available transcriptome data (RNA-Seq), qRT-PCR and Western blotting. In the adult murine brain, we found the highest Ngb-mRNA expression within the hypothalamic region of the brain stem, which was up to 20-fold higher than in the mouse cortex or the total brain. Other mammals like pig and vole also revealed elevated levels of Ngb expression in the hypothalamus. Based on these findings, we phenotypically analyzed a new conditional Ngb KO mouse model by performing a differential transcriptomic analysis of wild type and Ngb-deficient hypothalamic tissue (RNA-Seq). The high regional expression differences in the mammalian brain raise the question of distinct Ngb functions in highly and lowly expressing cell types. Our results can now generate new region-specific functional hypotheses and uncover previously unknown Ngb-dependent biochemical pathways and gene responses in the murine hypothalamus.
PP-NB-04

The chemically synthesized Ageladine A- derivative LysoGlow84 stains lysosomes in viable mammalian brain cells

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Based on the known chemical structure and chemical synthesis of the sponge alkaloid Ageladine A we synthesized the Ageladine A-derivative LysoGlow84. The two-step synthesis started with the Pictet-Spengler reaction of histamine and naphthalen-2-carbaldehyde to a tetrahydropyridine intermediate which was finally oxidized with activated manganese(IV) oxide to LysoGlow84. Structure and purity of the synthesized LysoGlow84 was confirmed by NMR spectroscopy and mass spectrometry. The fluorescence intensity emitted by LysoGlow84 depended strongly on the pH of the environment with highest fluorescence intensity recorded between pH4 and pH6 in the blue to green color range. The fluorescence maximum (330 nm excitation) was found at 440 nm.

Exposure of cultured rat brain astrocytes for hours to micromolar concentrations of LysoGlow84 did not compromise the cell viability as demonstrated by several viability assays. However, application of LysoGlow84 to astrocytes revealed promising properties of the compound to stain lysosomes. Conventional fluorescence microscopy (Leica and Zeiss) of LysoGlow84-treated astrocytes revealed a dotted cellular staining pattern and an almost perfect co-localization of LysoGlow84 fluorescence with that of Lysotracker Red®. By confocal scanning microscopy (Leica SP5) large overlaps in cellular fluorescence and only small differences in stained structures were observed, if the cells had been stained with both dyes. Thus, LysoGlow84 is a new promising dye that stains lysosomes in viable mammalian cells.

PP-NB-05

The Origin of Deuterostome Vision- Echinoderms Possess R-opsin AND C-opsin Expressing Photoreceptors Both Likely to be Involved in Vision.

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In our studies on echinoderm photoreceptors, we showed that a representative of this group of non-chordate deuterostomes, the purple sea urchin (Strongylocentrotus purpuratus), expresses a set of at least six different opsin genes, indicating a much higher organization level of photoreceptive systems than previously assumed. We demonstrated a rhabdomeric opsin expressing photoreceptor cell (PRC) type, (typical for visual PRCs of many protostomes), to form aggregated units potentially functioning in directional vision. Here we report on our findings on another opsin, Sp-Opsin1, the sea urchin equivalent of ciliary opsins, deployed in many chordate visual PRCs. We found c-opsin protein and RNA respectively, to be widely distributed within the sea urchin epidermis reminiscent of the often proposed overall “dermal light sense” of these animals. Interestingly, in our recent phylogenetic analysis sea urchin ciliary opsin clusters together with pinopsins, which are expressed in PRCs triggering a shadow response in Ciona larvae and in Xenopus tadpole larvae respectively. However, starfish and brittle star representatives show a completely different pattern of c-opsin protein distribution. In these groups, c-opsin epitopes were immunodetected exclusively in spines, whereupon in brittle stars this protein seems to be present in a cell type that might be involved in bioluminescence. Recent experiments in echinoid larvae (S. purpuratus) furthermore show ciliary opsin protein in locomotory cilia. Our data allows insights into the course of PRC evolution within echinoderms, with one c-opsin protein type potentially serving different visual functions. Our findings of a rhabdomeric AND a ciliary opsin type presumably involved in echinoderm vision, is also relevant for our understanding of eye evolution towards the vertebrate lineage.
**Neurobiology**

### PP-NB-06

**Is the size of postsynaptic depolarization correlated with area of synaptic contact?**

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The swimmeret system of crayfish is a model system to study coordination of distributed neural networks in locomotion. This system comprises a bilateral chain of four identical local networks, so called modules, with each driving the movement of one limb. Additionally two coordinating neurons project from each module and integrate through only one non-spiking Commissural Interneuron, ComInt1. ComInt1 always receives coordinating information from three ipsilateral located coordinating neurons. This information is integrated via a gradient in synaptic strength, in so far as the coordinating neuron from the posterior neighboring module elicits the strongest postsynaptic response and the others are successively weaker.

Here we present anatomical studies that characterize the morphological reason for the synaptic gradient in ComInt1. We recorded ComInt1 and axons of the coordinating neurons intracellularly and filled them with fluorescent dye. Afterwards we used immunohistochemical staining to mark synaptic regions. We demonstrated that ComInt1 extended small branches that run lateral to the coordinating axons. We could identify these branches as areas where synaptic transmission takes place, as synaptic regions are clearly visible. Three distinct pools of synaptic regions on ComInt1 have been identified (N=8). Our results further indicate that there is a statistical difference in the area covered by these pools of synaptic regions, as one of it is always larger than the other two. This may explain the gradient in synaptic strength measured intracellularly in ComInt1.

### PP-NB-07

**Adaptation of central lateral line units in the MON of goldfish (Carassius auratus) induced by double stimulation**

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The first site of sensory integration in the ascending lateral line pathway is the Medial Octavolateral Nucleus (MON). We studied adaptation of MON neurons in the goldfish Carassius auratus induced by double stimulation. We asked whether a first (reference) stimulus leads to a decrease in the response to a second (probe) stimulus. Stimuli were sinusoidal water motions (50Hz, 500 ms duration, rise/fall times 100 ms) generated by a vibrating sphere (8 mm diameter). In a first stimulus paradigm, the probe level was increased relative to the reference level (“second level tuning”), while the time interval between reference and probe was minimal (0 ms). In the second paradigm, the time interval between reference and probe was increased (“inter-stimulus-interval tuning”) while both stimuli had equal and constant levels. Neuronal responses were recorded extracellularly from single and multi units. Responses to the probe were reduced, i.e. discharge rates were decreased when reference and probe had the same level and inter-stimulus-interval was minimal, indicating response adaptation. This effect could be compensated for by a 5-10 dB increase of the probe level over the reference level or by an increase of the inter-stimulus-time-interval of about 500 ms. These results are similar to those reported for auditory neurons in the Inferior Colliculus of barn owls (Singheiser et al. 2012, Europ J Neurosci 35). Other studies from our lab and from the auditory system in cats (Huang and Buchwald 1980, Elec Clin Neurophysiol 49) indicate that adaptation to successive stimuli can occur already in primary afferent nerve fibers. This suggests that forward masking does not require substantial neuronal processing in the ascending sensory pathways.
PP-NB-08

Innervation patterns and synaptic connections of original and regenerated olfactory afferents in the antennal lobe of *Locusta migratoria*

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Locust antennal lobes (AL) contain microglomerular structures innervated by olfactory receptor neurons (ORN) from the antennae. We examine the relation between the annulus' position on the antenna and its representation in the antennal lobe, changes of a possible pattern during regeneration processes and the functionality of newly established synapses after regeneration.

Neurobiotin labelling of afferents from single antennal annuli that are present in the first larval stage are represented in a ring shaped pattern mostly in the outer areas of the AL while those that developed in later larval stages arborize more or less equally throughout the AL neuropil. After unilateral injury of the antennal nerve (axotomy), olfactory afferents regenerate into the AL, but apparently fail to reestablish the conspicuous ring shaped pattern and restriction to certain areas of AL neuropil. Regenerated fibers send processes everywhere in the AL and re-innervate the remaining glomerular structure.

Whether functional synapses are reestablished during regeneration is tested electrophysiologically. Directly after deafferentation, intracellular recordings from antennal projection neurons reveal complete absence of responses to odor stimuli (cis-3-hexenal) which elicit reliable responses in 40% of neurons on the untreated side. In adult locusts the number of responding neurons increases between day seven and 21 after deafferentation until the normal response level is reached. Interestingly, this process happens much faster in 5th instar nymphs where it takes only 10 days to regain the normal response level.

PP-NB-09

The influence of the endocannabinoid system on active avoidance learning in zebrafish (*Danio rerio*)

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The endocannabinoid system (ECS) is thought to be highly conserved among vertebrate species. Neurocytochemical findings have indicated that the main cannabinoid receptor 1 (CB1) is expressed in the medial pallium (MP) of the zebrafish dorsal telencephalon, a brain region, which is essentially involved in the formation of emotional-associative memory. In mice and rats, acute and chronic exposure to CB1 agonists and antagonists disrupts cognitive functions as well as emotional processes.

In this study we looked for a homologous effect in the zebrafish by use of an active-avoidance-learning (AAL) paradigm in a fish shuttle-box. In the shuttle-box the zebrafish were trained to recognize a red light as a conditioned stimulus to avoid a mild electric shock (aversive stimulus). The role of the ECS was investigated by using a receptor agonist (THC: Δ⁹-tetrahydrocannabinol) and an antagonist (Rimonabant).

After reaching the learning criterion of 45% avoidance reactions, we tested the influence of acute activation and inactivation of CB1 on retention of AAL. We found that neither THC nor Rimonabant impaired behavioral performance of zebrafish. However, chronic administration of CB1 agonist and antagonist had significant effects on AAL acquisition and storage in long term memory. As it has been shown in mammals, CB1 inactivation led to an improvement of acquisition learning, while chronic THC administration impaired acquisition learning.
PP-NB-10

Robustness of a rhythmic circuit to temperature changes

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Swimmerets are four pairs of limbs attached to the abdomen of crayfish used for forward swimming. They are controlled and innervated by four abdominal ganglia. In each hemiganglion a neuronal circuit drives motor neurons which activate the limbs in alternating cycles of power-stroke (PS) and return-stroke movement. An identified coordinating circuit synchronizes these central pattern generators, so that a metachronal wave is present where the last segment starts the PS and the anterior modules follow with a latency of 0.25. Here we wanted to test if the coordination is robust against temperature changes.

We used animals which were acclimated at normal temperature (14°C) and two extreme conditions: cold (4°C) and warm (25°C). All experiments were done on the isolated nervous system with extracellular recordings of PS nerves responsible for swimmeret movements. Saline with temperatures between 4°C to 35°C was perfused over the nerve cord. In all experiments the coordinating pattern remained stable with a phase lag of 0.25 between PS bursts. The period, burst duration, and PS burst strength statistically decreased with increased temperatures. Middle acclimated animals produced well coordinated rhythmic activity over a larger temperature range (4 to 25°C) than animals acclimated under extreme conditions. The coordinated rhythm of cold acclimated animals became unstable at around 15°C and broke down at high temperatures. Warm acclimated crayfish had very slow or uncoordinated rhythms at low temperatures, which became stable at temperatures between 18°C to 30°C. When motor neurons were recorded intracellularly at the same time, their membrane potentials hyperpolarized during warm and depolarized in cold conditions.

PP-NB-11

Innervation and transmitters of a centipede’s neuroendocrine system

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In comparison to insects, the neuroendocrine system of myriapods is far less understood. A closer look at neuroendocrine systems of arthropod species other than insects and crustaceans is worthwhile to gain insight into common morphological and functional principles. Until today, many questions are open. Those are e.g. where neurosecretory centers are located in the brain of myriapods, to which brain areas in insects they are comparable, if glands are homologous to insect neuroendocrine organs. We investigated the neuroendocrine glands, Gabe organs, of the centipede Lithobius forficatus with regard to innervations by the central nervous system and expressions of transmitters known from the insect neuroendocrine system. Our results will help to identify homologous parts within the neuroendocrine systems of arthropods.
A ciliary protein involved in motion vision gain control

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We describe an evolutionarily conserved cilium protein that is not only expressed in Drosophila ciliated cells, but also in one type of visual interneurons in the fly’s medulla. These interneurons are components of the visual motion detector but their relevance for motion vision is unclear. By testing for optomotor reflexes in mutants, we found that the protein is essential for motion vision. Motion vision was also abolished when we genetically suppressed the electrical activity of the interneurons, and the animals overestimated motion stimuli when the excitability of the neuron was thermogenetically increased. Follow-up experiments revealed that the interneuron type is a component of both the ON and OFF channels of the motion vision pathway that alters the motion vision gain. This defines an essential protein for fly motion vision, shows that conserved ciliary proteins can serve non-ciliary functions, and identifies the cellular component of the fly’s visual motion detector that controls the motion detector gain.

Descending modulation of motor activity in the stick insect

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The neuromodulatory substance octopamine plays an important role in insect locomotion. Octopamine is released from dorsal unpaired median (DUM) neurons. Six DUM neurons with somata located in the posterior part of the locust subesophageal ganglion have axons that are bilaterally descending (abbreviated DUM-SD) to thoracic ganglia (Bräunig and Burrows, 2004). We investigated the activity of presumably homologous neurons in the stick insect Carausius morosus during walking. Moreover, we studied whether DUM-SD neuron activity contributes to the modulation of motor activity in the mesothoracic ganglion. Using semi-intact preparations and intracellular recordings, we observed phasic depolarization of DUM-SD neuron membrane potential and the generation of action potentials during stance phases of the stepping middle leg. Mechanical stimulation by passive movement of legs was excitatory to DUM-SD neurons. In contrast, pharmacologically evoked activity in central pattern generating neurons (CPGs) had no effect. Thus, excitation of DUM-SD neurons during walking most likely arises from leg sensory organs rather than from coupling to CPG activity. In recent experiments, spike activity in DUM-SD neurons had two major effects on sensory-motor interaction in the mesothoracic ganglion. 1. Stimulation of some DUM-SD neurons decreased resistance reflex responses in middle leg extensor tibiae motoneurons (N=5). Reflexes were evoked by stimulation of the femoral chordotonal organ (fCO). 2. Spike activity in other DUM-SD neurons induced a general increase in extensor tibiae motoneuron activity during fCO stimulation (N=5).
PP-NB-13

A quest for excitation: Immunohistochemical analysis of a circuit for sensory gating in the ELL of *Gnathonemus petersii*

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The electrosensory lateral line lobe (ELL) of electric fish is the first processing stage of electrosensory information and has been analyzed for its functional connectivity, histology and immunohistochemistry in great detail (Bell 2005). However, there is a considerable gap between this wealth of data and the assumed functional connectivity: transmission from excitatory sensory afferents via granular cells, the relay elements of the ELL, has been found to be inhibitory, but all functional investigations call for excitatory elements in this path as well. Here we endeavor to shed light on this enigmatic lack of excitatory granule cells, using GABA, Glutamate and GAD immunohistological staining of the ELL of *Gnathonemus petersii*.

Due to double labelling with signal amplification based on the Streptavidin method in conjunction with anterograde identification of individual granule cells we have been able to localize GABA and Glutamate positive granule cells in deep and upper granule cell layers of the ELL. Thus our data supports the presence of previously hypothesized “on” channels in the sensory pathway of the ELL.

A second major lack of knowledge concerns the two functional somatotopic maps of the ELL. These are tuned to different features of the sensory input which, based on documented behavioral capabilities, need to be merged. To better understand the functional circuitry in both maps, we explore the degree of convergence of sensory input in both maps. This is currently being done for subclasses of neurons which enable us to determine the degree of convergence and to establish if processing in both zones occurs in a columnar fashion.

PP-NB-14

Brain centers involved in associative learning in the Zebrafish (*Danio rerio*) - measured by c-Fos expression

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Teleost fish are capable of successful associative learning. They can connect certain sensory stimuli to particular consequences, e.g. a food reward, mild electric shock or noxious stimuli. When investigating structures in the fish brain, relevant for learning, mostly lesion studies have been conducted that indicated an involvement of the telencephalic lateral pallium (Di) in spatial learning and the medial pallium (Dm) in emotional learning. These two areas have been proposed to be homologous structures of the mammalian hippocampus and amygdale, respectively. In the fish brain, they are known to be interconnected and to communicate during a learning process, but we assume that there are additional brain regions involved in learning, all of which constitute a memory formatting neuronal network.

In an appetitively motivated two-choice paradigm, Zebrafish were taught to discriminate between two colors, i.e. to avoid one color and approach another one. Learning was indicated by a significant increase of correct choices. After learning, the brains of the fish were investigated by immunohistochemical labeling of the c-Fos protein. The expression of the immediate early gene c-Fos is an indirect marker of neuronal activity because c-Fos is overexpressed when neurons become more active. In those Zebrafish that had successfully learned the discrimination task, we found an increase of c-Fos in the two pallial regions, Dm and Di. Currently we are investigating subpallial brain regions, e.g. the striatum, and certain nuclei of the diencephalon, e.g. the thalamus, posterior tuberculum and the hypothalamus.
PP-NB-15

3D fine structure and interconnections of cone pedicles in the European anchovy

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The teleost “outer” retina is characterized by a species-specific and geometrically regular pattern of cone photoreceptors. This pattern is maintained on the level of cone terminals in the outer plexiform layer (OPL) before these “pedicles” are wired with secondary order neurons in a convergent and/or divergent manner. A first step to disentangle OPL connectomics is done by describing the 3D fine structure of the cone pedicles with their horizontal pattern, cell-type specific radial stratification, number and arrangement of presynaptic ribbons, synaptic triads and not least with their inter-pedicular contacts.

Retinal fragments of the anchovy Engraulis encrasicolus from the Atlantic were processed for TEM and embedded in epoxy resin. The samples were sliced and imaged with a FIB/FESEM crossbeam workstation. The resulting image stack was reconstructed with Amira® performing manual segmentation and digital surface rendering.

We found that the staggered row pattern of anchovy polycones as well as the triple cone pattern of some retinal regions is retained on the pedicle level. A distinct radial displacement (ca. 1.5 µm) of long/lateral and short/central cone pedicles and ribbons can be revealed. Furthermore an unexpectedly dense interconnecting network of fine pediculare telodendrites with cone type specific 3D geometry (with and without synaptic structures) was uncovered.

Masked by the 3D entanglement and complete space filling of the pedicles with their fine protrusions and a multitude of secondary order neuron dendrites a well-ordered and cell-type specific wiring scheme emerges in the anchovy’s OPL. The telodendritic network indicates a strong lateral and directional modulation of downward signals among the photoreceptors.

PP-NB-16

Phonotactic flight behaviour and vertical sound source localization of the parasitoid fly Emblemasoma auditrix (Diptera: Sarcophagidae)

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Acoustically directed movement in a three dimensional space is a complex task performed notably by birds, bats and insect species. The precision of acoustic orientation depends on directionality of the hearing system as well as on the auditory behaviour. The fly Emblemasoma auditrix is a parasitoid of the cicada Okanaganana rimosa and locates its host in the complex habitat of a forest. Here we experimentally analysed the phonotactic flight behaviour of the fly especially in the vertical domain in the field. Acoustic targets were located already from resting positions. Firstly, the azimuthal orientation of a sound source was determined. Therefore the following phonotaxis by flight is predominately two-dimensional. The flight path is not linear, but somewhat S-shaped. Nevertheless, the vertical sound source positions are detected and the flies mostly landed above the loudspeaker. Different flight parameters have been analysed in respect to sound source position, sound pressure level and carrier frequency of the signal.
Neurobiology

PP-NB-17

Proliferation and plasticity in adult *Tribolium castaneum*

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With its fully sequenced genome and the susceptibility for reverse genetics based upon RNA interference (RNAi), the red flour beetle *Tribolium castaneum* is best suited to study development and plasticity of the nervous system. While plasticity can be provided by various mechanisms we focus on ongoing cell proliferation in the adult beetle brain. It is well established that neurogenesis persists in the mushroom bodies (MB) of adult insects, including the red flour beetle *T. castaneum* where neuroblasts giving birth to MB Kenyon-cells remain active for more than one month after adult eclosion (Zhao et al. 2008. Devel Neurobio, 68: 1487-1502). Using the 5-ethyl-2’-deoxyuridine (EdU) technique in combination with immunohistochemistry against various neuromediators - including e.g. neuropeptides and NO-synthase - and the glia-cell marker reversed-polarity, we labeled the progenies of adult persisting neuroblasts, determined their identity and counted the newborn Kenyon-cells in the first days after adult eclosion.

In several studies it was proposed that newborn neurons of MBs may play a role during olfactory processing and learning. Currently we combine the EdU-staining with olfactory stimulation using different odors like the leaf alcohol cis-3-hexen-1-ol or the beetle’s aggregation pheromone 4,8-dimethyldecanal (DMD), odor deprivation or knockdown of ORCO to investigate whether the rate of MB neurogenesis depends on olfactory input.

PP-NB-18

Using *Drosophila* to understand the evolutionarily highly conserved dopaminergic neurotransmission

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Key processes in physiological systems have been evolutionarily conserved from fruit flies to men. We employ *Drosophila melanogaster* as model to unravel the physiological and pathophysiological significance of dopaminergic neurotransmission. Dopamine is a neurotransmitter relevant for a great variety of physiological tasks and it is of central importance in various human neurological disorders. In *Drosophila*, four dopamine receptors with different expression patterns in the nervous system and cells of the digestive tract are known. GFP-based reporter systems were employed to identify environmental factors (stressors) that modulate dopaminergic neurotransmission. An in-depth transcriptomic study of isolated dopamine-producing cells from control animals as well as from stressed ones revealed signaling pathways involved in the response to these stressors. Moreover, we used the targeted expression of a temperature-gated ion channel (dTRPA1) to analyse the consequences resulting from a continuous stimulation of dopamine-receptor expressing cells. Findings from these experiments might be crucial to identify environmental factors that have beneficial or adverse effects for the development of e.g. Parkinson’s disease.
PP-NB-19

Short-term plasticity of orientation tuning in the optic tectum of adult zebrafish (*Danio rerio*)

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With its amenability to genetic approaches the larval zebrafish has been established as a model for a variety of research questions, including the investigation of visual neuronal processing.

We here focus on short-term plasticity in the optic tectum of adult zebrafish, induced by adaptation with pattern motion. While studies of the optic tectum of zebrafish or *Xenopus* larvae and the use of transgenic lines led to knowledge about neuronal short-term plasticity during development, little is known about the persistence of such effects in adult fish. To investigate plasticity in a fully developed brain, we deliver calcium sensitive dyes via electroporation to single neurons in physiologically defined tectal layers.

Our present studies aim to investigate if and how adaptation alters the tuning of orientation- or direction-specificity in single tectal neurons. In *Xenopus* larvae it is known that sensory experience can lead to plasticity (functional or structural) of tectal circuits and that modification of direction selectivity during development by visual input is possible (Podgorski 2012, Engert 2002). Furthermore, Dragoi (2001) showed an adaptation induced short-term plasticity in the visual cortex of cats.

For zebrafish we so far found that both, orientation- and direction-selectivity can be plastic following adaptation with pattern motion of fixed orientation and direction. Our preliminary findings indicate functionally relevant plasticity of tectal neurons in adult zebrafish, which leads to an enhancement of orientation- and direction-selectivity as well as to shifts in the tuning functions in a large fraction of neurons. We suggest that this type of plasticity might help accentuate responses to rapidly changing visual inputs.

PP-NB-20

Modulation of velocity tuning in the optic tectum of adult zebrafish (*Danio rerio*)

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Over the past years the zebrafish (*Danio rerio*) became an ideal model species for investigating visual processing and its underlying cellular mechanisms in the optic tectum. Ultimately, the optic tectum in fish is the central brain area where visual input is processed to direct motor response output. So far, studies concerning visual neuronal computations were mostly performed on zebrafish larvae.

As opposed to the use of genetic lines, we obtained data from wild type adult zebrafish. In order to find evidence for neural short-term plasticity in optic-tectal neural circuits we performed conventional wide field microscopy with calcium sensitive dyes to quantify single-neuron activity. Neurons in the optic tectum of fish have been found to respond to certain velocity ranges of pattern motion. Evidence for neuronal activity-dependent shifts in velocity tuning, which have been described for mammalian visual cortex, are still lacking in fish, and the correspondence yet need to be elucidated. Therefore, we started to investigate how different velocities of square wave grating patterns are processed in adult zebrafish optic tectum, and whether changes in velocity tuning can be effectively induced by applying distinct adaptation protocols.

So far, our data strongly indicates the presence of neuronal computations that can be modulated by adaptation protocols, inferring neural short-term plasticity in the optic tectum of adult zebrafish. We seek to provide veritable results, which help understand the underlying subcellular mechanism of neuronal processing in the optic tectum and elucidate the potential physiological relevance of neuronal short-term plasticity, including implications for associated locomotor behavior.
PP-NB-21

Visual acuity and learning in mudskippers (*Periophthalmus novemradiatus*)

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Indian dwarf mudskippers (*Periophthalmus novemradiatus*) are amphibic gobies (Gobiidae, Oxudercinae). They are active terrestrial predators and are highly alert to visual stimuli, even at a distance, where potential threats are spotted. Here we investigated the visual acuity of Indian dwarf mudskippers psychophysically in a two-alternative forced choice experiment, and obtained first insights into their learning abilities at the same time. In a first experiment, mudskippers were trained to discriminate between black-and-white vertical and horizontal stripe patterns (gratings) in air. Vertical stripe patterns were of variable width, while horizontal stripe patterns were as fine as to appear grey. Fish were trained to crawl or jump (on a thin water film with their eyes in air) into a decision chamber. At the far end of the decision chamber, one vertical and one horizontal grating were presented on the left and on the right; these positions were changed from trial to trial in a pseudorandom pattern. The animal had to choose and approach the vertical grating (S+) in order to receive a food reward. Decision distance was defined by a dividing wall between the stimuli and was 30.5 cm. Seven mudskippers were trained to the procedure. Six animals reached 80% correct choices within 4 to 7 sessions, five animals finished the experiment. Behavioural visual acuity was 1.26 ± 0.17 cycles per degree. In a second experiment, stimuli were presented at a larger distance. Mudskippers showed significantly reduced cooperation and learning success as compared to the first experiment. This might indicate that the animals did not accommodate to far away objects, or that they pay less attention to stimuli at a distance as long as stimuli are static.

PP-NB-22

Estimation of object distance based on electrosensory input dynamics

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The distance to an object is an important piece of information when navigating the environment. Behavioral test have shown that weakly electric fish like *Gnathonemus petersii* can detect and discriminate object distance, although such an estimation is not trivial without the availability of disparity information: These fish build up a 3-dimensional dipole field around their body by discharging impulsive signals from their electric organ. This electric field leads to a 2-dimensional current distribution across the animal’s skin, which holds an array of electroreceptors. Objects differing in their conductivity from that of water modulate the field, which is reflected in changes in the current distribution at the skin, which is termed the electric image (EI) of an object. Animals use the information contained in these EI to acquire sensory information. For a static electric scene, i.e. when analyzing single EI, EI cues such as image blur or relative amplitude measures (von der Emde, G.; et al.; Nature 1998) can serve to determine e.g. object distance. However such an analysis requires a detailed image analysis with a sufficiently high resolution that involves a high computational effort. Moreover the result needs to be interpreted with respect to body position to avoid ambiguities (Hofmann V.; et al.; J Phys Paris 2013).

In contrast to a static analysis, integrating electric flow information, that is the spatiotemporal input dynamics arising from relative motion, might facilitate information extraction. We here evaluate how electric flow analysis, e.g. temporal slope to amplitude ratio, temporal image shift (motion parallax) or the temporal change of EI amplitude enable the estimation of object distance.
Adaptive responses of peripheral and central lateral line units to repetitive dipole stimuli

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Adaptation is a common feature in all sensory modalities. Sensory systems can adjust their sensitivity to constant-amplitude stimuli and they decrease their neural output in response to repeated stimuli. We investigated whether peripheral and central lateral line units in the goldfish, Carassius auratus show sensory adaptation to repetitive stimuli. We presented sequences of 21 sine wave stimuli to the fish. Within a sequence the inter stimulus time intervals were kept constant. Stimulus sequences with long (2 s) and shorter (0.5 s, 0.1 s and 0 s) inter stimulus intervals were presented. Neuronal recordings were made from single fibers in the posterior lateral line nerve and from lateral line units in the medial octavolateralis nucleus.

The majority of both peripheral (57 %, n=213) and central lateral line recordings (54 %, n=169) showed a systematic decrease in the evoked discharge rate as function of stimulus repetition. For peripheral units, both the numbers of units, that exhibited a rate decrement and the percentage of rate decrement increased with decreasing inter stimulus time intervals. With 2 s inter stimulus time intervals 31 % (n=18 ) of the units showed a systematic decrease, whereas 50 % (n= 56) of the units showed a systematic decrease with 0.1 s and 0 s inter stimulus time intervals. For central lateral line units, we observed that 23 % (n= 43) of the units showed a systematic decrease in firing rate with 2 s inter stimulus intervals. With inter stimulus intervals of 0.5 s and 0.1 s 17 % (n= 23) and 26 % (n= 26) of the units showed a decreased firing rate.

The data show that both peripheral and central lateral line units adjust their neural output as a function of stimulus repetition. This is comparable to what has been reported for peripheral and central auditory neurons in mammals. Future studies will show whether the effects described here are indicative of habituation, i.e. sensory learning.

Neuroprotective effects of erythropoietin in insects

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Besides its role in vertebrate erythropoiesis, the cytokine erythropoietin (Epo) has been recognized as a signal that mediates adaptive cellular responses triggered by diverse stimuli in various non-hematopoietic tissues including the nervous system. We hypothesize that these general tissue-protective functions of Epo evolved before its hematopoietic function in vertebrates. Hence, we have studied potential beneficial effects of human recombinant Epo (rhEpo) on cell cultures derived from nervous systems of several insect species. RhEpo increased the survival of primary cultured neurons from Locusta migratoria exposed to apoptosis-inducing conditions and accelerated the regeneration of neurites. We demonstrated that rhEpo-induced protection of cultured locust neurons involves cellular mechanisms that interfere with apoptotic pathways. Both, the type of receptor and the activated intracellular signaling pathways are similar to those described in mammalian nervous systems. Similar studies on Drosophila melanogaster suggested that Epo-mediated protection is absent in this highly derived insect species. To expand our study to other insect species we are using primary brain cell cultures of Tribolium castaneum and of Gryllus bimaculatus, as well as a permanent CNS-derived cell line from Manduca sexta. The identity of cell types within each culture system, their relative contribution to the overall cell number in culture, as well as their viability under different combinations of treatment with rhEpo and inducers of apoptosis are currently investigated. The studies on nervous tissue from different species will provide information whether Epo-mediated neuroprotection is a common characteristic among insects.
PP-NB-25

**Induction of learning through thermogenetic activation of Kenyon cell ensembles in Drosophila**

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Drosophila represents a model organism to analyze neuronal mechanisms underlying learning and memory. Kenyon cells of the Drosophila mushroom body are required for associative odor learning and memory retrieval. But is the mushroom body sufficient to acquire and retrieve an associative memory? To answer this question we have conceived an experimental approach to bypass sensory input and to thermogenetically activate sparse and random ensembles of Kenyon cells directly. We found that if the artificial activation of Kenyon cell ensembles coincides with a salient, aversive stimulus learning was induced. The animals adjusted their behavior in a subsequent test situation and actively avoided reactivation of these Kenyon cells. Our results show that memory retrieval is characterized by a closed feedback loop between a behavioral action and the reactivation of sparse ensembles of neurons.

PP-NB-26

**Neuronal Circuit Dissection in the Drosophila Brain: the Role of Serotonin-Releasing Neurons in Arousal**

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For animals, an optimized arousal state is essential for motivating or inhibiting specific behaviors. A wide range of genes, hormones, peptides and biogenic amines contribute to the regulation of the arousal level. In this study the potential modulatory effect of serotoninergic neurons on arousal in *Drosophila* was explored using a thermogenetic approach. We show that thermoactivation of serotoninergic neurons using the dTRPA1 channel led to a significant reduction in walking velocity. This locomotory effect can be distinguished from a deficit in the movement ability. In addition, we tested the fly’s response in startle induced negative geotaxis, feeding and courtship assays and found that thermoactivation of serotoninergic neurons lead to the significant reduction in animal’s response to different external stimuli. Overall, these data provide evidence for a neuromodulatory effect of serotonin on arousal in *Drosophila*.

In order to dissect the neuronal circuits underlying modulation of arousal we used stochastic and intersectional gene expression approaches and demonstrate that a subset of serotoninergic neurons in the PMP cluster is involved in the regulation of arousal in *Drosophila*.
**Temporal processing of odor stimuli from Olfactory Receptor Neurons to Projection Neurons**

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Olfactory Receptor Neurons (ORNs) respond to odor stimuli with temporal patterns of activity that depend on odor identity, odor concentration and receptor type. We performed measurements of the activity of single ORNs in *Drosophila* and analyzed these temporal patterns taking in account a precise quantification of the time dependent stimulus. We found that the dynamics of the odor stimulus itself depends on odor identity and concentration and that it strongly modulates the ORN response. We were able to assign a single response function to a single ORN and to predict the response of this ORN to two different odors solely from measurements of the stimulus. We also found that ORN adaptation capabilities maintain response dynamics remarkably similar across a large range of stimulus and background intensities (Martelli, 2013). These results suggest that ORN response properties enable the olfactory system to capture information about stimulus independently from its intensity. In order to investigate this hypothesis we developed a computational model of the *Drosophila* Antennal Lobe (AL), the first synaptic processing center of olfactory information. Our simulation includes three neuron populations: ORNs, inhibitory Local Neurons (LN) and output Projection Neurons (PNs). The model reproduces known static properties of the AL (the divisive normalization, Olsen 2010) while providing temporal resolution on the activity of the single neurons. We investigate how dynamical properties of the input ORN activity affect the encoding of single odorants and odor mixtures in the population of PNs.

**Non-visual functions of visual opsins**

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Evidence is accumulating that opsins can sense more than light. In *Drosophila*, opsins were recently implicated in larval temperature preference behaviours and in hearing in adult flies. Here, we report that *Drosophila* larvae also require visual opsins for locomotion, and show that the proprioceptors that control locomotion do express opsins. Opsin mutant larvae crawling in darkness displayed locomotion defects, including reduced distance travelled and reduced locomotion speeds. When we genetically rescued the function of the respective opsin gene, normal locomotion was restored. Opsin-dependent locomotion defects associated with altered temperature preference behaviours and closely resembled the locomotion deficits of mutants whose chordotonal neurons are impaired. Promoter-fusions revealed that opsins are expressed in the serially arranged, proprioceptive chordotonal neurons in the larval body wall. Opsin expression was confirmed with antibodies, and chordotonal neurons seemed to be the only neurons that express opsins outside the larval eye. This suggests that larval locomotion and temperature preferences might converge on chordotonal neurons. It also strongly supports the idea that light-independent opsin functions evolutionarily predated their use as photoreceptor proteins.

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**PP-NB-29**

**TRPML, a new TRP for hearing**

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Several members of transient receptor potential (TRP) family channels have been reported to play roles in *Drosophila* hearing, including NOMPC (~TRPN1), the TRPVs Nan and lav, and the TRPAs Pyx and Pain. Here we report that fly hearing also involves TRPML mucolipin channels, mutations in which are implicated in the neurodegenerative disorder mucolipidosis type IV (TRPML1) in humans and hearing impairments in Varitint-Waddler mice (TRPML3). The latter hearing defects arise from a point mutation that render TRPML3 constitutively active, yet hearing remains uncompromised by the complete loss of this channel, leaving the auditory relevance of TRPMLs elusive. Promoter fusions revealed that *Drosophila* TRPML is expressed in auditory sensory cells, as TRPML3 in mammals. Null mutation of TRPML diminished mechanical amplification and sensitive sound-responses, whereby normal hearing was partially restored when we expressed TRPML rescue constructs in the mutants. To assess the relative placement of TRPML in the auditory pathway, we generated mutants that lack TRPML along with the TRPV lav, which forms heteromultimeric channels with Nan to negatively regulate the mechanical amplification gain. The amplification gain values lay between those from null mutants of TRPML and those from lav mutants, suggesting that TRPML operates in parallel to Nan-lav in amplificatory gain control. This establishes a role of TRPML channels in normal hearing and implicates yet another TRP in the function of *Drosophila* auditory sensory cells.

**PP-NB-30**

**Are DUM-neurons involved in local auditory processing of the bush-cricket Ancistrura nigrovittata?**

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Auditory processing in insects was mostly studied in sensory cells, intersegmental neurons projecting into the brain and very few large local neurons. In the prothoracic ganglion, the first level of auditory processing, frequency dependent inhibition, directional and temporal inhibitions have been found. The only well characterized local neuron, the Omega-neuron (homologous to crickets’ON1), produces strong directional inhibition¹. Previous data compiled on a bush-cricket demonstrate the likely importance of other local neurons for frequency and also temporal processing. For example, frequency dependent inhibition in intersegmental neurons has been found, which may rely on groups of DUM-cells². Several DUM-cells exist, directly responding to acoustic stimuli³. Physiological characterization of DUM-cells is done using intracellular electrophysiology, the cell can then be stained for morphology.

DUM-cells exhibit a huge variety of frequency tuning: from cells responding only to vibratory stimuli to cells responding best in the audio or in the ultrasonic range and cells exhibiting clear IPSPs at certain frequencies. DUM-cells may be involved in temporal inhibition, since blocking Cl- channels with picrotoxin changes the response of temporally selective neurons like AN2, and since first immunohistochemical data indicate that at least part of the auditory DUM-cells contain the transmitter GABA.

¹Molina and Stumpner, 2005. JEZ 303A:1085
²Stumpner, 1997. JEB 200:1089
PP-NB-31

The Dopamine type 2 receptor (D2R) contributes to consolidated memory.

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Memories are classified as consolidated (stable) or labile according to whether they withstand amnestic treatment, or not. In contrast to the general prevalence of this classification, its neuronal and molecular basis is poorly understood. Here, we used the Drosophila aversive odor-learning paradigm to separate between the impacts of a type-2 dopamine receptor (D2R) on anesthesia-resistant (ARM) or anesthesia-sensitive memory (ASM). We report that mutation of D2R results in specific loss of ARM without impacting on ASM performance. Genetic rescue localized D2R function to Kenyon cells (KCs) of the mushroom bodies, an important memory center in the fly brain. Since KCs have previously been shown to require the type-1 dopamine receptor dDA1 for support of ASM and ARM, our data suggest that memory components are dissociated at level of dopamine receptors.

PP-NB-33

Characterization of a neuroligin 2 knock-out in Drosophila melanogaster

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Neuroligins are postsynaptic adhesion molecules which mediate synaptic formation and maturation. Mutations in neuroligins have been associated with Autism spectrum disorders (ASDs) and neuroligin-deficient mice have been found to show abnormal social behaviour that resembles ASD symptoms.

In an earlier study, we were able to show that Drosophila lacking Drosophila neuroligin 2 (dnl2) exhibit altered courtship, mating and aggression behaviour (Hahn et al., 2013). We found three major differences between dnl2 KO flies and wildtype flies: Firstly, dnl2 KO flies show more and faster movements. NMR spectrometry revealed elevated NAA and lactate levels, which point to a higher neuronal activity, which corroborates our ethological findings. Secondly, the mutant strains showed diminished centre avoidance, which was previously also attributed to mutations in the mushroom bodies. Immunostaining for dnl2 reveals a clear localization to the mushroom bodies. Lastly, group behaviour is strongly altered in the mutant strains. Dnl2 KO flies avoid groups twice as often as their wildtype conspecifics. Additionally, within a group they show a wider inter-individual distance.

**PP-NB-34**

**Phenotypic synaptic plasticity in the brain of the nectar-feeding ant *Camponotus rufipes***

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In the polymorphic nectar-feeding ant *Camponotus rufipes*, media sized workers undergo an age-dependent division of labor: young ants stay in the nest in complete darkness for 4-6 weeks and then start to forage utilizing visual and olfactory navigation and orientation capabilities. Their foraging activity is influenced by the respective season: In summer, workers are nocturnal foragers while they switch to diurnal foraging behavior in winter. Using age-marked, dark-reared workers, we aim to investigate neuronal plasticity in the mushroom bodies (MB), high-order sensory integration centers which are implicated in learning, the formation of associative memories and orientation. Using synapsin-immunolabeling for whole-mount brains, we combined volume measurements with the quantification of synapsin-positive presynaptic boutons of the calyx, the MB main input region. In particular, we investigated how the effect of age and the exposure to light act on the synaptic composition in the olfactory and visual calyx subregions, the lip and the collar. The results indicate that the volume and the density of synaptic boutons of both calyx subregions increase within a period of six weeks in total darkness. In addition, we furthermore found that the density of MG decreased significantly in the collar, but not in the lip after exposing dark reared ants to light for four days similar to the results in *Cataglyphis fortis* and the honey bee (Stieb et al. 2010, 2012; Dev Neurobiol; Scholl et al. 2014; Dev Neurobiol). These results indicate that the structural plasticity is preliminary driven by visual experience and might play an important role in the timing of behavioral transitions from nursing to foraging. Supported by DFG SFB 1047 “Insect Timing” (B5).

**PP-NB-35**

**The neuropeptide SIFamide regulates feeding and appetitive behavior in *Drosophila***

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The central nervous system monitors the systemic energy balance and adjusts the animals’ behavior according to environmental cues and internal needs. Recent studies have advanced our knowledge about molecular and cellular mechanisms that coordinate changes in the neuroendocrine and neuromodulatory state of the fly according to its metabolic needs, promoting or inhibiting discrete feeding behavioral subprograms (Pool & Scott, 2014). During recent years various neuromodulatory systems could be assigned to the food intake behavior of *Drosophila*. Many of these signaling systems appear to be functionally conserved throughout evolution. One of the neuropeptides being strongly conserved throughout invertebrate evolution is the neuropeptide SIFamide (Verleyen et al., 2009). The exact function of this neuropeptide is still not known. The all-embracing innervation patterns of the four SIFamidergic neurons, however, suggest a neuromodulatory function in many different aspects of signal processing. Through artifical induction of neuronal activity of SIFamidergic neurons through the heat-inducible cation channel dTRPA1, we could show that increased SIFamide release leads to enhanced odor driven appetitive behavior, increased food intake and higher sensibility to sugar in a proboscis extension reflex paradigm. In a continuative step we combined two independent bi-partite expression systems (Gal4/UAS and LexA/LexA:AOP) to anatomically and physiologically identify the neuronal circuits of discrete SIFamide related behavioral feeding subprograms. First results will be presented.
PP-NB-36

**Origin of high variability in colony odor representation in the ants’ brain**

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Ants can discriminate nestmates (NM) from non-nestmates (nNM) and react aggressively against the latter. Discrimination is based on odors that consist of many different chemical components on the ants’ body (cuticular hydrocarbons: CHCs). Although colony odors vary only in the ratios of the components, ants are able to recognize amazingly fast and precise whether an encountered ant belongs to nestmates or non-nestmates. Importantly, colony odors vary over time, depending on diet and environment. Repeated stimulation with NM colony odors revealed a significantly higher variability of spatial response patterns in the antennal lobes compared to repeated stimulations with nNM colony odors [1]. The functional significance of this neuronal variability is unknown. We addressed the question where this variability in representation origins from.

Therefore, we analyzed the response behavior of single functional units in the antennal lobe (glomeruli) over consecutive NM and nNM colony odor stimulations. The total number of glomeruli that are only activated by NM odors and not by nNM odors (NM specific glomeruli) is not different to the total number of nNM specific glomeruli. Interestingly, only when NM colony odors are presented repeatedly, some glomeruli respond in addition to the previously as NM-specific identified glomeruli, and this was not the case for repeated nNM colony odor presentation.

Such plasticity in representation of colony odors may promote discrimination of NM and nNM colony odors, particularly when colony odors change over time. However, the mechanism how this plasticity is generated within the neuronal network remains to be investigated.


PP-NB-37

**A model for temperature-dependent locomotion in Drosophila melanogaster**

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Temperature is one of the most important environmental factors for all animals, and it is crucial for an organism's survival to detect not only preferred temperatures, but most importantly to avoid noxious temperatures. This is especially true for small ectotherm animals such as the fruit fly *Drosophila melanogaster*, which therefore needs an efficient thermosensory system. Recently the field of insect thermosensation has been gaining momentum: Using genetically accessible organisms such as *Drosophila*, many new transduction molecules and sensory organs were identified in the last years. However, *Drosophila* thermosensation research still lacks a null model, which predicts the ability to move in different ambient temperatures and identifies the cold-trapping temperature range. Therefore, we investigated the temperature-dependent walking behaviour of *Drosophila* using neuroethological methods. In particular, we study how the locomotion of fruit flies depends on the ambient temperature, and whether this is affected by different rearing temperatures.
Impact of hypoxic conditions on the innate immune system - from Drosophila melanogaster to chronic inflammatory diseases of the lung

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The innate immune system represents an ancient host defense mechanism that protects against invading pathogens in vertebrates and invertebrates likewise. Important immune effector molecules to fight microbial infections are antimicrobial peptides (AMPs). In the fruit fly Drosophila melanogaster, induction of AMP gene expression was thought to depend exclusively on NF-κB transcription factors via activation of two highly conserved signaling cascades, the IMD and the Toll pathway. With respect to bacterial or fungal infections this concept is undisputed. However, recent studies show that AMP expression in Drosophila can also be achieved independently of the canonical NF-κB signaling pathways and in the absence of a pathogen challenge. One of these non-microbial activators of innate immunity is hypoxia. It occurs particularly during chronic inflammatory disorders e.g. of respiratory organs, where it has a direct effect on the barrier epithelium. Depending on the duration and severity, hypoxia can lead to cell injury and death, and consequently organ injury and failure. From our and other groups’ research, we know that hypoxic stress fulfills a crucial function in various inflammatory reactions of the epithelia in general. Our intention was to shed light on the connecting mechanisms between hypoxia and innate immunity with focus on the respiratory organs of Drosophila melanogaster as a model system. We succeeded in identifying the forkhead box transcription factor FoxO as a novel activator of a hypoxia-induced AMP response, and detected a hitherto unidentified pathway of FoxO mobilization independent of the conventional FoxO elicitors.
Impact of temperature on the serine proteases of *Daphnia pulex*

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Protein digestion in the intestine of the zooplankton crustacean *Daphnia pulex* is mainly accomplished by serine proteases of the chymotrypsin and trypsin type. 64 potential functional extracellular serine proteases could be identified in the genome of *Daphnia pulex* in our study. The genes of these digestive proteases were mainly down-regulated under acute heat stress, while they were mostly up-regulated with increasing temperature under continuous exposure to 10°C, 20°C and 24°C. Enzymatic activity of trypsin and chymotrypsin was measured using photometric assays. Additionally, enzymatic function was assessed subsequent to electrophoretic protein separation by activity staining in acrylamide gels. Results revealed a change between high trypsin activity in the cold to high chymotrypsin activity at increased temperatures. The increase of staining intensity in activity gels along with rising temperatures confirms these results. Proteases with molecular weights between 22 kDa and 85 kDa could be identified, the highest staining intensity was observed in the range between 35 and 44 kDa. Moreover, there was a gain in the number of serine protease isoforms with increasing temperature. These acclimation processes to varying temperatures are supported by the diversity due to the expansion of the serine protease gene family.

Basic functional studies of murine epithelia cell lines for the use in CF research

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**Question:** Human primary epithelial cell cultures and immortalized cell lines are a common tool for the study of the underlying defects in the frequent human genetic disease Cystic fibrosis (CF). However, their use is highly limited in functional studies and therefore, new cell lines need to be validated for electrophysiological measurements. In this study the established murine cell lines mSEC1-CF (S498X mutation) and mSEC1-nonCF were analyzed for the use in transepithelial current measurements for a project (GALENUS), involving functional CFTR correction by Sleeping Beauty (SB)-based CFTR vectors and CFTR-specific Zinc Finger Nucleases (ZFN).

**Methods:** First, we performed genotyping by PCR analysis of DNA extracts from mSEC1-nonCF and mSEC1-CF cells to assure correct genotype expression. Afterwards, we analysed the functional phenotype by electrophysiological measurements in Ussing chamber experiments. We characterized the CFTR secretion in the cells using a CFTR activating cAMP cocktail and the CFTR specific inhibitor CFTRinh172. Furthermore, we identified the CFTR protein expression by Western blot analysis.

**Results:** The genotyping analysis showed that both cell lines express the right genotype and the electrophysiological Ussing chamber measurements demonstrated the appropriate functional phenotype for both cell lines. Moreover, we were able to identify a suitable antibody to detect CFTR in mouse cells.

**Conclusions:** The murine cell lines mSEC1-CF and mSEC1-nonCF cells reflect the CF and nonCF situation and are well suitable for the ongoing studies using the two novel strategies (SB and ZFN) for a long long-term therapeutic transgene expression.
PP-P-05

**Functional characteristics of phosphoglucone-isomerase (PGI) isoforms in *Daphnia magna* and their role in thermotolerance**

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Aquatic animals have to cope with a continuously changing environment since abiotic conditions like temperature change seasonally and also on a daily base. The phosphoglucone-isomerase (PGI) is an important enzyme in glycolysis and gluconeogenesis as it catalyses the reversible isomerization of fructose 6-phosphate and glucose 6-phosphate. Its role for thermotolerance is discussed. We analyzed clones of the aquatic crustacean *Daphnia magna*, which express different isoforms of PGI. These are characterized by 1-5 amino acid exchanges of the PGI enzyme, which are located on the surface. The active site remains unchanged, but the position of the c-terminus at the interface of PGI dimers changes variably. Analysis of enzymatic function by photometric activity assays revealed significant differences in the reaction rate and the substrate affinity of different isoforms. The Michaelis-Menten parameters are also affected by acclimation and measuring temperatures. There is no difference in the activation energy or the specificity constant of the enzyme, which is a measure of its catalytic efficiency. However, the isoforms differ in terms of their thermostability. These differences in PGI enzyme parameters may be important for the animals’ physiological performance in their habitat. Therefore, microevolutive processes affecting the abundance of the PGI gene loci might enable *Daphnia* populations to cope with different temperature conditions.

PP-P-06

**Functional CFTR restoration due to mRNA transfection and its influence on ENaC expression**

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Cystic fibrosis is caused by genetic defects in the *cystic fibrosis transmembrane conductance regulator* gene (CFTR), which encodes for a cAMP-regulated chloride channel located in the apical membrane of polarized epithelial cells. It is proposed that CFTR influences other ion channels, e.g. the epithelial Na⁺ channel ENaC. In the forefront, we successfully restored Cl⁻ secretion in human nasal epithelial (HNE) cells using optimized wtCFTR-mRNA. We assessed the minimal mRNA concentration that is needed for a proper functional CFTR expression and determined the persistence of the CFTR expression. Therefore, we carried out transepithelial Ussing chamber measurements after transfection of HNE cells with wtCFTR-mRNA. The amount was reduced stepwise in order to determine the minimal concentration that is needed for the most efficient restoration of the functional Cl⁻ secretion. Furthermore, the duration of the CFTR expression was evaluated 24, 48 and 72h after transfection. Immunofluorescence and biochemical approaches were performed to support the data. Functional experiments revealed an increase in the cAMP-stimulated CFTR current 24 and 48h after wtCFTR-mRNA transfection in HNE cells. This current could also be inhibited by the specific blocker CFTRinh₁₇₂. Reduction of mRNA resulted in a functional Cl⁻ secretion that is comparable to higher concentrations. In addition, we observed a decrease in the ENaC current, probably due to the effect of increased CFTR expression. The successful restoration of the CFTR function could be sustained even by a reduction of the wtCFTR-mRNA concentration and over a time period of 48h. The decrease in the amiloride sensitive current suggested that CFTR transfection could also regulate ENaC channel expression.
PP-P-07

Development of an immunological strategy to combat the poultry red mite *Dermanyssus gallinae*


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The Poultry Red Mite (PRM) *Dermanyssus gallinae* causes high economic losses and is the most devastating parasite in poultry farming worldwide. Different chemical, physical and biological strategies try to control the expansion of PRM. However, a solution to this problem still has the highest priority. Therefore, an immunological strategy is presented here, which could allow the protection of chicken from PRM. The first step of the development consisted in the isolation of proteins from PRM. Following, chicken were immunized with different PRM extracts. Then, IgY were extracted from eggs of the immunized chicken and a PRM *in vitro* blood-feeding assay was performed. This assay, which used fresh chicken blood spiked with IgY isolated from the differentially immunized animals enabled the detection of antibodies which led to PRM mortality.

In the next step, individual proteins were isolated through 2D gel analysis combined with antibody analysis and used for a second immunization of chicken. Analysis through ELISA and western blots showed a high specific antibody production against PRM extracts. Subsequently, it was possible through proteomics to identify specific proteins as candidates for the production of antibodies which display anti-RVM activity. These results suggest a high potential of this strategy for the development of a vaccine against the poultry mite *Dermanyssus gallinae*.

Key words: economic losses, immunization, poultry protection, vaccine technology

PP-P-08

Functional analysis of the vertebrate globin repertoire in cell culture

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Hemoglobin (Hb) and myoglobin (Mb) have well-established functions in oxygen transport and storage. In recent years, six additional globin types (androglobin [Adgb], neuroglobin [Ngb], cytoglobin [Cygb], globin E [GbE], globin X [GbX] and globin Y [GbY]) have been identified in vertebrates. These “novel” globins share the common characteristics of globins, e.g. the typical globin fold and the ability to bind oxygen (O₂). The functions of these globins are still poorly understood. In addition to specific roles in O₂ supply, several hypotheses have been put forward. These putative functions include a role in intracellular signaling, decomposition of nitric oxide and the detoxifying of reactive oxygen (ROS).

ROS are highly reactive derivatives of oxygen (e.g. H₂O₂, OH· or O₃), which can cause damage to the cells by oxidation of DNA, proteins or (membrane-) lipids. We employed a strictly comparative approach in a cell culture system. Mouse neuronal cells (HN33) were stably transfected with zebrafish (*Danio rerio*) Mb, Ngb, Cygb 1 and 2, or GbX, with mouse (*Mus musculus*) Mb or Ngb, or with chicken (*Gallus gallus*) GbE. HN33 cells transfected with the empty vector (pcDNA3.1+) were employed as mock-control. The cells were kept under hypoxic conditions (1 % O₂ for 6, 24 and 48 h) and ROS-stress was induced by hydrogen peroxide (H₂O₂) for 24 h. Cell viability was measured via the activity of the mitochondrial dehydrogenases and the ATP-amount of the cells. The results suggest that all globins enhance cell viability under hypoxia with variable efficiency and that GbX and Ngb most efficiently protect from ROS. In addition, we measured globin-induced ROS-production, which showed that Ngb and GbE increased ROS under hypoxia and H₂O₂-induced stress. These results allow evaluating different hypothesis of globin functions.
Physiology

PP-P-09

Pleiotropic functions of Allatostatin A-expressing cells in coordinating physiology and feeding behaviour in Drosophila

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Allatostatin A peptides are implicated in the control of insect feeding in Drosophila, and are expressed in central and peripheral neurons and enteroendocrine cells. Using fly genetics, behavioural and physiological assays, we investigated the pleiotropic functions of AstA cells and tried to dissect functional subgroups. We show that activation of specific subsets significantly reduces feeding, but at the same time also affects fly locomotor activity. Other subsets of AstA cells appear to influence defecation behaviour and midgut motility.

Our results reveal that different subsets of AstA cells are involved in several interrelated processes to promote metabolic and behavioral adaptability to internal and environmental requirements. Our findings also provide evidence for a functional role of AstA-expressing enteroendocrine cells that is not limited to the regulation of gut physiology.

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PP-P-10

The globin gene repertoire of the lamprey: Convergent evolution of hemoglobin and myoglobin in jawed and jawless vertebrates

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Agnathans occupy a key phylogenetic position for illuminating the evolution of vertebrate anatomy and physiology. Evaluation of the agnathan globin gene repertoire can thus aid efforts to reconstruct the origin and evolution of the globin genes of vertebrates, a superfamily that includes the well-known proteins hemoglobin and myoglobin. We have characterized the globin repertoire of the sea lamprey (Petromyzon marinus) and the Arctic lamprey (Lethenteron camtschaticum). We identified 23 globin genes in the sea lamprey, and 18 full length and five partial globin genes in the Arctic lamprey. Phylogenetic analyses showed that agnathan and gnathostome hemoglobins are not orthologous, but emerged independently from a globin ancestor. In the sea lamprey, distinct hemoglobins are differentially expressed in larvae and adults, suggesting a hemoglobin switch analogous to that in the gnathostomes. Both lamprey genomes harbor two copies of globin X, but, surprisingly, no ortholog of neuroglobin. We identified a cytoglobin in the lampreys, which is the only orthologous vertebrate-specific globin of gnathostomes and agnathans. Two globins were highly expressed in muscles of P. marinus, thus representing functional myoglobins. Phylogenetic analyses indicate that these heart-specific globins are not orthologous to the myoglobins of Gnathostomata, indicating that specialized respiratory proteins for oxygen transport in the blood and oxygen storage in the striated muscles evolved independently in both lineages.
PP-ZS-01

A new and critically endangered species of Onychophora (Peripatidae) from a vulnerable biodiversity hotspot - the Brazilian savannah

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In recent years, a high number of endemic species may have gone extinct silently in the biodiversity hotspot Brazilian savannah (=Cerrado), as researchers are not able to describe them as fast as their natural habitats are being destroyed. This especially holds true for onychophorans, or velvet worms, which include many point endemic species and, in this biome, are restricted to small gallery forests susceptible to disturbances. In the present study, we have used a combined morphological and molecular approach to investigate a relict and critically endangered undescribed species of Onychophora form the Cerrado. Our morphological analyses using light and scanning electron microscopy revealed novel characters and character states for Peripatidae, such as six interpedal structures per segment, modified scales on the genital and posterior body region of females, and a third class of dermal papillae, herein named peripheral accessory papillae. Moreover, our findings show that this species belongs to a new genus, as it exhibits a unique set of morphological characters inconsistent with the diagnoses of other described genera of Peripatidae. In addition to morphological data, the new taxon is also supported by karyotypic, slime protein profiling and molecular data, including sequences of the mitochondrial genes COI and 12S rRNA. Unfortunately, data collected in situ suggest that this species is prone to an extremely high risk of extinction in the wild, as it only occurs in three small, unprotected fragments of forest (0.47-0.67 km²), which are being progressively affected by human activities. According to the IUCN Red List categories and criteria, this species is classified as Critically Endangered and requires urgent conservation efforts.

PP-ZS-02

Analysis of Hox genes in the water bear Hypsibius dujardini (Tardigrada)

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The Hox genes are crucial for patterning the antero-posterior (a/p) body axis during development of bilaterally symmetric animals. These genes are a group of highly conserved, homeobox-containing transcription factors that usually lie in a cluster in the genome and show spatial colinearity, i.e. they are expressed in the same order along the a/p body axis as they appear in the cluster. To clarify the Hox repertoire in Tardigrada, one of the three major groups of Panarthropoda (Onychophora + Tardigrada +Arthropoda), we sequenced and analysed the transcriptome of the eutardigrade Hypsibius dujardini. Our transcriptomic and phylogenetic analyses revealed homologs of only five expressed Hox genes, including labial, Deformed, Hox3, fushi tarazu, and Abdominal-B. Interestingly, we identified three copies of Abdominal-B in our transcriptomic data, suggesting that they are functional genes. These findings correspond well with our searches in the recently released genome sequences of H. dujardini. Our ongoing studies will clarify whether the identified Hox genes lie in a cluster in the tardigrade genome and whether their expression patterns in embryos of H. dujardini resemble those of onychophorans and arthropods.
**PP-ZS-03**

**Morphological evolution and robust species identification with the rotifer genus *Synchaeta*.**

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*Synchaeta* represents an important, cosmopolitan genus of rotifers comprising over 40 known species. However, species within the genus are difficult to identify and distinguish from one another using existing morphological information from the literature because the diagnostic features used are often either highly subjective or are subject to preparation artifacts. Surprisingly, despite the accepted species-specific nature of the rotifer mastax, its morphology has, to date, hardly been used in the context of species identification in *Synchaeta*. As a first step towards rectifying this situation, we investigated the morphology of six species of *Synchaeta* using a combination of light and scanning electron microscopy and describe a set of universal, more objective characters from both the habitus and mastax. We show that characters of the habitus and the trophi morphology are indeed suitable for species identification and differentiation within the group. Furthermore, we examine the evolution of these characters, and of the species within the genus, in a phylogenetic framework as obtained from these morphological data and, additionally, from DNA sequence information as well. In so doing, we highlight the correlation between specific character states and ecological traits including feeding and swimming behavior. In the future, we will expand our investigations to include more species of *Synchaeta* and thus to provide an increasingly comprehensive overview of the genus.

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**PP-ZS-04**

**Seroprevalence of *Toxoplasma gondii* infection in women population in Sebha city, Libya**

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Toxoplasmosis is caused by the parasite *Toxoplasma gondii* and constitutes a major economic and public health problem in the world especially in the developing countries including North of Africa and Middle East. Few studies have been conducted on the prevalence of Toxoplasmosis in Libya. The test of this disease can be performed on a woman prior to or during a pregnancy to determine if she has been recently exposed to *Toxoplasma gondii*. There are two types of *Toxoplasma* antibodies may be presented in the blood: IgM and IgG. The present study aimed to determine the prevalence of Toxoplasma immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies in sera. from collected from 11 medical Centers in Sebha city, Libya. One hundred and seventy-four blood samples were collected from women during the period of April 2013 to March 2014 and screened for IgG/IgM antibodies by the Vitek Immuno Diagnostic Assay System (VIDAS) TOXO IgG and VIDAS TOXO IgM- avidity tests. The prevalence of IgG antibody in the study population was 53.5% (93/174), while examination of specific IgM antibody was negative in the all tested samples. The highest prevalence was recorded in Al-Qudes (86.4%) medical center and the lowest prevalence was found in Al-Kahera (31.3%) medical center, with no significant differences were observed between the different medical centers (P≥0.05). Also serological results involved a comparison in the infection prevalence among months of the year, the highest prevalence were recorded in February, November and July (80.0, 76.9%, and 72.2% respectively). In addition, the statistically significant differences were observed between the prevalence in months were noted (P
Mitochondrial phylogeography of grass snakes (*Natrix natrix*, *N. megalopechala*) conflicts with taxonomy and reveals an additional unexpected contact zone in Central Europe


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Grass snakes (*Natrix natrix*) represent one of the most widely distributed snake species of the Palaearctic region. Within *N. natrix*, up to 14 distinct subspecies are regarded as valid. In addition, some authors recognize big-headed grass snakes from western Transcaucasia as a distinct species, *N. megalopechala*. Based on phylogenetic analyses of a 1984-bp-long alignment of mtDNA sequences (cyt b, ND4+tRNAs) of 410 grass snakes, a nearly range-wide phylogeography is presented for both species. Within *N. natrix*, 16 terminal mitochondrial clades were identified, most of which conflict with morphologically defined subspecies. These 16 clades correspond to three more inclusive clades from (i) the Iberian Peninsula plus North Africa, (ii) East Europe and Asia and (iii) West Europe including Corso-Sardinia, the Apennine Peninsula and Sicily. Hypotheses regarding glacial refugia and postglacial range expansions are presented. Holocene range expansions led to the colonization of more northerly regions and the formation of secondary contact zones. In Central Europe, there are two contact zones of three distinct mitochondrial clades, one of these contact zones was theretofore completely unknown. In agreement with previous studies using morphological characters and allozymes, there is no evidence for the distinctiveness of *N. megalopechala*. Therefore, *N. megalopechala* is synonymized with *N. natrix*.

Phylogeography and population genetics of the Mediterranean tortoise *Testudo hermanni* (Gmelin, 1789)

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*Testudo hermanni* or Hermann’s tortoise is a medium-sized, terrestrial species spread over the Mediterranean area of the Iberian, Apennine and Balkan Peninsulas. After years of confusion within the taxonomy, *Testudo hermanni* is now recognized as a species with the two subspecies *T. h. hermanni* and *T. h. boettgeri*. Until now the population differentiation within *T. h. boettgeri* is unclear and some authors have suggested that the western Croatian populations might represent a distinct taxon. First genetic studies with mitochondrial markers do not sustain this new third subspecies. Population differentiation was studied using 19 polymorphic microsatellite markers of more than 260 individuals from the whole distribution area. The results support the already recognized taxonomy with both subspecies *hermanni* and *boettgeri*. Within each subspecies several genetic clusters were detected indicating a high genetic diversity. Furthermore, our microsatellite data do not confirm the existence of a third subspecies.
Species identification using protein mass spectrometry: First investigations across metazoan diversity

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During the last decades, matrix assisted laser desorption/ionization (MALDI) -time of flight (TOF) mass spectrometry (MS) was found out to be a fast and reliable method to discriminate species based on the analysis of protein mass spectra. Until now, this approach was mainly used to identify microorganisms (e.g. pathogens), while only a few metazoan taxa were already tested (e. g. mosquitos, biting midges, selected fish species and scorpions ). In order to test the reliability and abilities of this proteome-based species identification approach across a broad range of metazoan taxa, we include many different marine groups from the North Sea (e.g. fish, mollusks and crustaceans), as well as terrestrial invertebrates in our study. A preliminary study revealed promising results for selected flatfish species (Pleuronectiformes) from the North Sea. These findings confirm the usefulness of proteome mass spectra for species identification based on MALDI-TOF MS.

New giant-sized stick insects reported from an unexpected Madagascan radiation

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Madagascar is one of our planet’s leading biodiversity hotspots exhibiting an extraordinarily distinct and diverse, but also severely endangered biota. Many elements of its fauna are poorly documented and remain largely unexplored. For example, the evolutionary history of the Malagasy stick insects (order Phasmatodea) is entirely obscure. Stick insects form at least four endemic taxonomic groups on Madagascar, i.e. families, subfamilies and tribes, with numerous taxa awaiting formal description. Here we report two new spectacular members of the Achriopterini, giant-sized stick insects exceeding 20 cm body length. Achriopterini comprises Achrioptera with a handful of species and the monotypic Glawiana. Traditionally, Achriopterini were considered to be a subgroup of Phasmatinae with affinities to the Orientalic Stephanacridini. Large size and strong dimorphism were interpreted as plesiomorphic traits of Achrioptera and a middle to late Jurassic origin of the Achriopterini was proposed, when Madagascar was still connected to India, Antarctica and Australia. However, our phylogenetic analysis of nuclear (28S, H3) and mitochondrial (COI, COII) genes recovered Achriopterini as a young and subordinate taxon among Phasmatodea, closely related to the Anisacanthidae, another endemic Malagasy lineage of stick insects, and thus suggests that large size and strong sexual dimorphism must be derived conditions in Achrioptera.
Context-dependent signaling of sky-compass cues in an insect brain

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The polarization pattern of skylight can provide a compass cue that is used for allocentric orientation by various insect species, e.g. to steer a steady course during long-distance migrations. Studies in the desert locust *Schistocerca gregaria* have identified pathways of polarization-sensitive neurons which converge in the central complex of the brain. The central-complex network involves neurons tuned to the electric field vector (E-vector) of polarized light with preferred E-vector angles varying along slices of neuropils in a compass-like fashion (polarotopy).

We strive to elucidate how activity in this polarotopic population is modulated and possibly integrated with representations of additional cues to control compass-guided locomotion. Here, we report on two phenomena suited for this task: 1.) the adaption of responses to stationary E-vector angles and 2.) their disadaptation by salient events of visual motion.

Adaptation may correlate with the tendency to steer a steady course which was observed in tethered flying locusts. Responsiveness to varying E-vectors that, under a natural sky, indicate changes in heading direction was generally preserved but more variable at the output stage of the network. The disadaptation of responses by events of sudden small-field motion against the visual background might serve to prepare for spatial re-orientation in the near future, e.g. following escape maneuvers in response to approaching objects. Together, these processes can serve for a context-dependent signaling of compass cues as they may link polarization-signaling to behavioral goals and to critical events in the visual scenery.
OP-SSI-02

Integration of polarization and chromatic cues in the sky compass of the desert locust

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Like other migratory insects, the desert locust likely uses a sky compass mechanism for spatial orientation. In the sky, several cues, including direct sunlight, the polarization pattern of the sky and the chromatic and intensity gradient can be exploited for compass orientation. Previous work showed that the central complex in the locust brain holds a polarotopic internal representation of celestial E-vectors and may, therefore, act as an internal sky compass. It is generally assumed that E-vector detection alone does not provide unambiguous compass information because it does not differentiate between solar and antisolar directions. We therefore examined whether polarization-sensitive (POL) neurons of the central complex receive additional input providing information on the chromatic gradient of the sky. The intensity gradient of long wavelengths (green light) and the uniform distribution of short wavelengths (UV-light) across the sky lead to a chromatic gradient with highest intensity difference between long and short wavelengths near the sun and smallest difference in the antisolar hemisphere. We tested the responses of central-complex to zenithal polarized light and a green and UV-light spot rotating at an elevation of 45° around the head of the locust. All POL neurons tested were sensitive to the unpolarized stimuli. In most POL neurons the azimuthal tunings to both light spots were in the same position or close to each other. The difference between the preferred position of the green light spot and the preferred E-vector orientation was dependent on the neuron type. Furthermore the intensity of green light seems to influence the tuning, suggesting that the intensity gradient itself has an important role.

OP-SSI-03

The role of octopamine and tyramine receptors in honey bee division of labor

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Honey bees are famous for their complex division of labor. Although genetically closely related, some bees take care of the brood, while their sisters provide the entire colony with pollen (proteins) and nectar (carbohydrates). How a bee decides for a specific task is not known. It is generally believed that differences in sensory response thresholds lie at the basis of division of labor. As biogenic amines are important modulators of behavior, they are top candidates for regulating division of labor through sensory response thresholds. This talk will focus on the role of octopamine and tyramine and their receptors in honey bee division of labor.
OP-SSI-04

**Nutritive and sweet reinforcement is mediated by distinct dopaminergic cells in the *Drosophila* brain**

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Hungry flies can be trained to associate an odour with a sugar reward. This appetitive memory decays quickly, if the used sugar tastes sweet but contains no nutritive value (i.e. arabinose). If the sugar can be metabolized into energy (like sucrose), a single, two minute-long pairing of odour and reinforcing sugar leads to long-lasting memory.

We’ve previously shown that the sweet-only reinforcement is relayed by an octopamine signal that activates dopaminergic (DA) cells expressing the alpha-adrenergic receptor oamb (Burke, Huetteroth et al., Nature 2012). These cells belong to a cluster of ~140 DA cells in the anterior medial brain that together innervate all zones of the horizontal lobe of the mushroom body. Blocking these cells during memory acquisition abolished sucrose memory after 24 hours, so in this DA cluster both sweet and nutritive reinforcing signals seem to be integrated.

By combining morphological characterization, genetical manipulation and olfactory conditioning we sought to further characterize the food-related reinforcing properties of the DA cells in this cluster. We found subsets of DA cells that were required and sufficient for short-lasting memory only, and we found distinct DA cells that were required and sufficient for 24 hour appetitive memory. Artificially implanted memory by direct stimulation of these subsets revealed hunger state-dependency for only some cells. Together, our results support our previous findings and imply a high degree of labelled-line information flow for different reinforcing signals as a major component of learning and memory formation in the fly.

OP-SSI-05

**Pleiotropic functions of Allatostatin A-expressing cells in coordinating physiology and feeding behaviour in *Drosophila***

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Allatostatin A peptides are implicated in the control of insect feeding in *Drosophila*, and are expressed in central and peripheral neurons and enteroendocrine cells. Using fly genetics, behavioural and physiological assays, we investigated the pleiotropic functions of AstA cells and tried to dissect functional subgroups. We show that activation of specific subsets significantly reduces feeding, but at the same time also affect fly locomotor activity. Other subsets of AstA cells appear to influence defecation behaviour and midgut motility.

Our results reveal that different subsets of AstA cells are involved in several interrelated processes to promote metabolic and behavioral adaptability to internal and environmental requirements. Our findings also provide evidence for a functional role of AstA-expressing enteroendocrine cells that is not limited to the regulation of gut physiology.
**OP-SSI-06**

The Ion Transport Peptide, ITP, and the Pigment-Dispersing Factor, PDF, are the main peptides controlling locomotor activity rhythms in the fruit fly *Drosophila melanogaster*

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The clock network of *Drosophila melanogaster* expresses various neuropeptides, but a function in clock-mediated behavioral control was so far only found for the neuropeptide Pigment Dispersing Factor (PDF). Here we show that the Ion Transport Peptide (ITP), which is expressed in only two clock neurons per brain hemisphere plays a role in the control of the fly’s activity rhythms. Immunocytochemical analyses show that ITP, just like PDF, is most probably released in a rhythmic manner at projection terminals in the dorsal protocerebrum. This rhythm continues under constant dark conditions indicating that ITP release is clock controlled. ITP expression is reduced in the hypomorph mutant *Clk AR*, suggesting that the ITP expression is regulated by CLOCK. Using a genetically encoded RNAi construct we knocked down ITP in the two clock cells and found that these flies show reduced evening activity and increased nocturnal activity. Overexpression of ITP with two independent *timeless-GAL4* lines completely disrupted behavioral rhythms, but only slightly dampened PER cycling in important pacemaker neurons, suggesting a role for ITP in clock output pathways rather than in the communication within the clock network. Simultaneous knockdown of ITP and PDF made the flies hyperactive and almost completely arrhythmic under constant conditions. Under light-dark conditions the double-knockdown combined the behavioral characteristics of the single-knockdown flies. In addition, it reduced the flies’ sleep. We conclude that ITP and PDF are the clock’s main output signals that cooperate in controlling the flies’ activity rhythms.

**OP-SSI-07**

Living at different latitudes: the role of *Drosophila* I-LNv in setting the phase of the evening activity peak.

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The genus *Drosophila* contains over 2000 species that populate very different environments, from the equator to frigid zones. To survive in these diverse areas they have evolved specific behaviors to avoid being exposed to midday heats at lower latitudes or morning chill at higher ones. Moreover, differences in locomotor activity seem to correlate with specific features of their circadian clock neuronal network (Bahn et al., 2009; Kauranen et al., 2012; Hermann et al., 2013). Species of lower latitudes show a bimodal activity pattern, with a morning and an evening activity peak, and express the circadian photoreceptor CRY and the neuropeptide PDF (involved in the output of the clock) in both subsets of ventral Lateral Neurons (small and large LNv). On the other hand species of higher latitudes are mainly active in the second part of the day and show a reduced (if any) PDF expression in the small LNv (s-LNv) and no CRY expression in the large LNv (l-LNv).

We wondered whether differential CRY and PDF expression in the I-LNv of higher latitudes species compared to lower latitudes ones is directly responsible for their ability to better adjust to very long days and cooler temperatures, conditions they would be exposed to in a natural environment.

We used *D. melanogaster* and manipulated CRY and PDF expression in the I-LNv of higher latitudes species compared to lower latitudes ones is directly responsible for their ability to better adjust to very long days and cooler temperatures, conditions they would be exposed to in a natural environment.

We used *D. melanogaster* and manipulated CRY and PDF expression in the brain to simulate clock neurons properties of higher latitudes species. Interestingly doing this we could provoke a behavior typical of Northern *Drosophila* species in a species adapted to low latitudes environments.

We could also show that the I-LNv have a prominent role in setting the phase of the evening activity peak at the right time of the day.
OP-SSI-08

Olfactory projection neuron pathways in marine Isopoda and Remipedia

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The anatomy of the olfactory projection neuron pathway has been studied in several representatives of Malacostraca. Nevertheless, the morphology of olfactory projection neurons and second order olfactory neuropils are discussed rather controversially in Peracarida. Therefore, we investigated the olfactory projection neuron pathway of the marine isopods Saduria entomon and Idotea emarginata, using focal dye injections of the lipophilic tracer DiI into the olfactory neuropil. Both arms of the olfactory globular tract form a chiasm in the center of the brain and the olfactory projection neurons innervate the medulla terminalis and the hemiellipsoid body of the ipsi- and the contralateral hemisphere. Both protocerebral neuropils are innervated to a similar extent. This is reminiscent of the situation in the basal decapod taxon Dendrobranchiata. Thus, we propose that an innervation by the olfactory globular tract of both the medulla terminalis and the hemiellipsoid body is not only part of the decapod ground pattern, but also of the ground pattern of Caridoida. This proposal is based on the similar arrangement in Dendrobranchiata and Isopoda. The recent finding of hemiellipsoid bodies in certain Peracarida suggests a wider distribution of hemiellipsoid bodies in Malacostraca than previously thought. Chemoneuroanatomical data regarding cAMP signaling in the olfactory projection neuron pathway of Remipedia, a key taxon in the debate of tetraconate phylogeny, are considered in an evolutionary context.

OP-SSI-09

The olfactory pathway of T. castaneum and its peptidergic plasticity

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With the fully sequenced genome, its susceptibility for transgenetic approaches such as directed gene expression and powerful reverse genetics based on systemic RNA interference, and its longevity, T. castaneum offers an excellent system to study plasticity of the olfactory system. A prerequisite to understand changes in the olfactory pathway occurring either naturally or which are induced by manipulation is a thorough analysis of the system. By means of electron raster microscopy, immunostainings, backfills of the antenna and maxillary palps, and 3D reconstructions, we characterized the morphological features of the olfactory pathway. In addition we used RNA-sequencing to detect and raw localize the expression of olfaction relevant molecules, e.g. ORS, GRs, IRs, OBPs, SNMPs. The antennal lobes (ALs) of T. castaneum house 28 neuropeptides mainly located in local interneurons. By means of mass spectrometry, immunocytochemistry and 3D reconstruction we studied cellular distribution and relative changes in concentration for selected AL neuropeptides during the first days of adulthood. In summary, our findings suggest that regulation of neuropeptides during the early postmetamorphic phase depends on the perception of odor signals and thus favors an activity dependent mechanism for the maturation of the peptidergic system in the ALs.

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**OP-SSI-10**

**Descending modulation of motor activity in the stick insect**

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The neuromodulatory substance octopamine plays an important role in insect locomotion. Octopamine is released from dorsal unpaired median (DUM) neurons. Six DUM neurons with somata located in the posterior part of the locust subesophageal ganglion have axons that are bilaterally descending (abbreviated DUM-SD) to thoracic ganglia (Bräunig and Burrows, 2004). We investigated the activity of presumably homologous neurons in the stick insect *Carausius morosus* during walking. Moreover, we studied whether DUM-SD neuron activity contributes to the modulation of motor activity in the mesothoracic ganglion. Using semi-intact preparations and intracellular recordings, we observed phasic depolarization of DUM-SD neuron membrane potential and the generation of action potentials during stance phases of the stepping middle leg. Mechanical stimulation by passive movement of legs was excitatory to DUM-SD neurons. In contrast, pharmacologically evoked activity in central pattern generating neurons (CPGs) had no effect. Thus, excitation of DUM-SD neurons during walking most likely arises from leg sensory organs rather than from coupling to CPG activity. In recent experiments, spike activity in DUM-SD neurons had two major effects on sensory-motor interaction in the mesothoracic ganglion. 1. Stimulation of some DUM-SD neurons decreased resistance reflex responses in middle leg *extensor tibiae* motoneurons (N=5). Reflexes were evoked by stimulation of the femoral chordotonal organ (fCO). 2. Spike activity in other DUM-SD neurons induced a general increase in activity in *extensor tibiae* motoneurons during fCO stimulation (N=5).

**OP-SSI-11**

**Phenotypic synaptic plasticity in the brain of the nectar-feeding ant *Camponotus rufipes***

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In the polymorphic nectar-feeding ant *Camponotus rufipes*, media sized workers undergo an age-dependent division of labor: young ants stay in the nest in complete darkness for 4-6 weeks and then start to forage utilizing visual and olfactory navigation and orientation capabilities. Their foraging activity is influenced by the respective season: In summer, workers are nocturnal foragers while they switch to diurnal foraging behavior in winter. Using age-marked, dark-reared workers, we aim to investigate neuronal plasticity in the mushroom bodies (MB), high-order sensory integration centers which are implicated in learning, the formation of associative memories and orientation. Using synapsin-immunolabeling for whole-mount brains, we combined volume measurements with the quantification of synapsin-positive presynaptic boutons of the calyx, the MB main input region. In particular, we investigated how the effect of age and the exposure to light act on the synaptic composition in the olfactory and visual calyx subregions, the lip and the collar. The results indicate that the volume and the density of synaptic boutons of both calyx subregions increase within a period of six weeks in total darkness. In addition, we furthermore found that the density of MG decreased significantly in the collar, but not in the lip after exposing dark reared ants to light for four days similar to the results in *Cataglyphis fortis* and the honey bee (Stieb et al. 2010, 2012; Dev Neurobiol; Scholl et al. 2014; Dev Neurobiol). These results indicate that this structural plasticity is preliminary driven by visual experience and might play an important role in the timing of behavioral transitions from nursing to foraging. Supported by DFG SFB 1047 “Insect Timing” (B5).
OP-SSII-01

Role of epigenetics in insect development and immunity

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Gene expression in eukaryotes is regulated by histone acetylation/deacetylation, an epigenetic process mediated by histone acetyltransferases (HATs) and histone deacetylases (HDACs) whose opposing activities are tightly regulated. The acetylation of histones by HATs increases DNA accessibility and promotes gene expression, whereas the removal of acetyl groups by HDACs has the opposite effect. On the other hand, microRNAs are small non-coding RNAs mediating regulation of gene expression in eukaryotes at the post-transcriptional level. The talk addresses the role of histone acetylation and micro-RNAs in epigenetic control of transcriptional reprogramming in insects during development, wounding and infection. Using model insects such as the red flour beetle Tribolium castaneum and the greater wax moth Galleria mellonella we explored how histone acetylation and micro-RNAs mediate gene regulation during metamorphosis, stress adaptation and host-parasite coevolution. In addition, the presentation highlights the development of insect models as “early warning tools” and “whole-animal-high-throughput-systems” for the detection of trans-generational side effects of drugs on complex parameters such as fecundity, longevity and gender ratio and the epigenetic mechanisms behind.

OP-SSII-02

A Brave New Microcosm: how biological rhythms are reshaping our understanding of epigenetic mechanisms

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Epigenetic modifications such as DNA methylation and histone acetylation are important molecular events that act to regulate gene transcription. The general assumption is that epigenetic events are permanent. Biological rhythms, such as daily or seasonal rhythms are associated with extensive genomic plasticity in a diverse range of tissues and cellular phenotypes. Using a seasonally breeding species, this presentation will demonstrate light and hormone dependent regulation of DNA methylation in the adult hypothalamus. One genomic region in particular, Type 3 deiodinase (DIO3) is a target of the light induced changes in DNMT1/3B expression. Remarkably, the proximal promoter region exhibits reversible DNA methylation. These data highlight that epigenetic modifications are not necessarily permanent or heritable; but dynamic and plastic. Next generation sequencing of genomes and transcriptomes has also provided the ability to obtain an enormous amount of information at a molecular and cellular level. RNA-seq, in particular, led to the identification of non-coding RNA (ncRNA); both short and long sequences whose function is under considerable investigation. Seasonal changes in ncRNA suggest that the nucleus is a microcosm of molecular excitement. The study of biological rhythms in ncRNA will be a fascinating and daunting adventure for the next generation of scientists.
OP-SSII-03

Getting Started with Epigenetics: New Tools and Services

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DNA is wrapped around an octamer of histone proteins to form chromatin, then packaged into chromosomes by other structural proteins. The organization of genetic material in the nucleus has profound effects on all processes that require access to DNA. These processes must be tightly regulated, i.e. by epigenetics to maintain the integrity of the genome. Epigenetics refers to functionally relevant and heritable modifications to the genome that do not involve a change in the nucleotide sequence. These modifications are dictators to the "openness or the state of compaction" of the chromatin structure and ultimately influence gene regulation.

Much of the regulation of the genome involves the histone proteins that are the core of chromatin and chromosome structure. Histone modifications (e.g. acetylation, methylation, phosphorylation of histone tails) and DNA methylation at CpG islands are part of the mechanisms by which genome function is regulated. Protein effectors or readers recognize and bind to specific histone modifications. Similarly, the MBD family of proteins bind methylated stretches of DNA and initiate important interactions with other chromatin-associated proteins that lead to downstream regulatory effects.

Active Motif offers services and tools to study these important events through the development of high-quality products centered on chromatin biology and epigenetics:
- Epigenetic ChIP-Seq Services for genome wide data generation and analysis
- Chromatin IP kits and reagents
- Kits to study DNA methylation and histone modifications
- High-quality ChIP-validated antibodies to histones and chromatin proteins

The presentation will focus on ChIP-Sequencing, its challenges and how you can utilize our expertise and research tools to overcome them. You do not need to become an expert yourself in the many scientific and bioinformatic techniques needed to reproducibly generate high-quality, interpretable data with ChIP. Additionally the presentation will provide an overview on how Active Motif’s tools and reagents will help to establish epigenetic research in your own laboratory.

OP-SSIII-01

Perspectives on Biotechnological Improvements to the Sterile Insect Technique (SIT)

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SIT is a successful genetic pest management strategy to prevent, control, suppress, or even eradicate invasive insect pest species from islands, large agricultural production areas, or even complete continents. SIT is a species-specific and ecofriendly insect birth control measure involving mass production, sterilization, and sustained area-wide release of large quantities of sterilized insects. This leads to unproductive matings, which shrinks the population. Our current biotechnological efforts improve on transgenic female-specific lethality systems to enable more efficient sexing for male-only releases, on reproductive sterility systems to overcome the problem of radiation-reduced fitness, and on transgenic markers to better monitor the efficacy of SIT applications.

Most established transgenic sexing and reproductive sterility systems are based on the binary tTA expression system that can be suppressed by adding tetracycline to the food. However, to create ‘redundant killing’ in order to evade resistance development to such transgenic approaches, an additional independent conditional expression system is required. Here we present a perspective on the use of a second food-controllable binary expression system - the inducible Q system - that could be used in combination with site-specific recombinases to generate independent transgenic killing systems. We propose the combination of an already established transgenic embryonic sexing system to meet the SIT requirement of male-only releases based on the repressible tTA system together with a male-specific reproductive sterility system based on a spermatogenesis-specifically expressed homing endonuclease activated by Q-system controlled site-specific recombination.

A combination of completely independent transgenic sexing and reproductive male sterility systems, which do not share any active components and mediate the induced lethality by completely independent processes, would meet the ‘redundant killing’ criteria for suppression of resistance development and could therefore be employed in large scale long-term suppression programs using biotechnologically enhanced SIT.
More efficient, more safe: An attract and kill strategy using carbon dioxide combined with biocontrol agents for belowground herbivore pests

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Belowground pest control gained center stage in several agricultural production systems in recent years, causing both quantitative and qualitative damage and paving the way for secondary bacterial or fungal infections. Control options targeting these pests are limited, due to the phasing out of effective insecticides; new control options are therefore desperately needed.

The larvae of soil dwelling insects use carbon dioxide gradients, established by growing roots, to find their host plants. This long distance orientation cue is complemented by additional specific volatile cues emitted by the plants, resulting in a final acceptance of a host plant for feeding.

The “attract and kill” strategy (A&K) turns this strategy upside down: instead of bringing the control agent to the larvae, they are attracted to the control agent by combining these organisms with capsules emitting CO₂. When near to these capsules, mortality of belowground larvae significantly increases because of contact with the control agent. To make this strategy work under field conditions, however, the capsules need to fulfill several prerequisites.

Lab experiments demonstrated that several larval species were highly attracted to these artificial CO₂-capsules, but moved away when realizing that they were cheated. Additional compounds incorporated into these capsules, however, increased attractiveness and duration of stay.

We tested these capsules combined with either an isolate of an entomopathogenic fungus, Spinosad, or with a botanical insecticide in field experiments; in potato fields application of the A&K capsules resulted in significantly lower tuber damage in most, but not all fields, depending on the treatment schedule.

Necessary improvements of the A&K strategy for a standardized application routine in the field are currently tested in experiments this year at several locations. The implementation roadmap for this strategy will be discussed.

iBeetle: Genome wide RNAi screen for embryonic and metamorphic development in the beetle Tribolium castaneum

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Most of what we know about the genetics of arthropod biology is based on work in Drosophila melanogaster. However, several features of the higher Dipterans are derived, hard to study due to technical reasons or not representative for insects. Therefore, most of what we know about the evolution of gene function stems from a species, which in many respects is evolutionarily derived. In addition, not all gene functions have been identified due to redundancies by lineage specific gene duplications or gene loss. In the meanwhile, a number of alternative insect model systems are being established.

Tribolium castaneum stands out due to well established transgenic techniques, a strong and systemic RNAi response and a sequenced genome. In order to overcome current limitations in insect science, we are performing a large scale RNAi screen called “iBeetle”. First, we want to identify missing components of well studied processes independently of the Drosophila paradigm (e.g. segmentation). Further, we want to open new fields of insect research to functional analysis (e.g. stink gland biology; development of holometabolous insect diversity during metamorphosis). Finally, we aim at developing Tribolium into a complementary screening platform to identify genes that will be studied back in Drosophila or other model systems (e.g. lethal genes for pest control, adult muscle development).

The iBeetle project will generate approximately 9.000 data sets, which will foster basic research and insect biotechnology because it provides novel information on gene function complementing the information derived from Drosophila. The systemic nature and the ease of application of RNAi make Tribolium a screening platform for the identification of genes for processes that for technical reasons may be hard to screen for in Drosophila or the many emerging model organisms.
**OP-SSIII-04**

The peritrophic matrix: a potential target site for insect control

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Insect control is essential to prevent losses in agriculture and to minimize health risks due to biting insects transmitting various diseases. Chemically diverse insecticides have been developed over the past decades that, however, act on a comparably narrow spectrum of target molecules. In addition, increasing problems with insecticide resistance requires the search for alternative target sites. One interesting target site for insect control is the midgut epithelium, because several bacterial endotoxins have been shown to disrupt its physiological function. The midgut epithelium of most insects is lined with the peritrophic matrix (PM), which consists primarily of chitin fibrils and associated proteins (PMPs). The PM is thought to facilitate digestion and provide protection from abrasive food particles, enteric pathogens and secreted toxins. We examined the physiological roles of chitin synthase 2 (TcCHS2) and eleven peritrophic matrix proteins (TcPMPs) of the red flour beetle, Tribolium castaneum. While TcCHS2 is expressed along the entire midgut, the TcPMP genes are differentially expressed along the length of the midgut epithelium in feeding larvae. RNAi for two specific TcPMP genes resulted in depletion of fat body, growth arrest, molting defects and mortality. In situ permeability assays after oral administration of different-sized FITC-dextrans demonstrated that the knock-down of these TcPMPs, as well as TcCHS2, leads to the disruption of the PM’s anterior-to-posterior permeability gradient, which is supposed to facilitate digestion. The results from our study provide new insights into the physiological functions of PMPs in T. castaneum, and may help to establish new target sites for insect control.

**OP-SSIII-05**

Identification of lethal genes in *Tribolium castaneum* for application in pest control

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In recent years, the pollen beetle Meligethes aeneus has become an important pest of oilseed rape in Europe. The application of insecticides for controlling this pest resulted in increasing levels of resistance. To manage these problems alternative control methods are necessary. One emerging possibility is the use of transgenic plants, which express dsRNA against essential pest genes. Upon feeding on the plant, RNA Interference (RNAi) knocks down the gene and thereby kills the pest. This requires the identification of host sensitive target genes. In our work we use the red flour beetle *Tribolium castaneum* as a screening platform to identify such genes. Due to the systemic RNAi response, the simple keeping conditions and the large scale RNAi screen iBeetle, where thousands of novel genes are analyzed, *Tribolium castaneum* is an excellent model organism for this aim.

Of more than 5,000 genes analyzed in the iBeetle screen, and subsequent RNAi analysis, 11 most lethal genes were identified in *Tribolium*. Titration and injection of different dsRNA concentrations resulted in a mortality rate of 100 % on day six after injection and thereby showed a higher lethality compared to known targets. These genes did not share GO terms but their Drosophila orthologs were expressed in the central nervous system and carcass. Furthermore, we found three proteins with divergent sequences in several insects which might be useful for designing species specific dsRNA constructs. Combining treatments against combinations of these genes led to additive but not to synergistic effects.

RNAi has a great potential to control pest insects. Here we report 11 highly efficient targets for RNAi in pest control where some of them seem to be fast evolving in insects. Ongoing investigations of dsRNA ingestion in pollen beetle will reveal the potency of these genes in a pest species.
**OP-SSIII-06**

**CRISPR/Cas9 mediated genome editing in *Tribolium castaneum***

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The CRISPR/Cas9 system has rapidly revolutionized genome editing in the last two years. Originally derived from a bacterial defense system against invading DNA, it has been modified to edit genomes of a number of model species, including *Drosophila*, zebrafish, mouse and human cell lines [1]. The system relies on the expression of the endonuclease Cas9 and a short guide RNA that interacts with Cas9 and the target sequence, thus mediating the specificity [2]. Since this interaction relies on correct base pairing, finding and predicting target sequences is straightforward and does not require any libraries or complicated cloning steps.

We present our approach to adapt the CRISPR/Cas9 system to the red flour beetle *Tribolium castaneum*. While most approaches in other models rely on RNA synthesis, we drive both the expression of both the short guide RNA and Cas9 with endogenous promoters. Thus, our easy-to-use system can be modified to express any guide RNA with only one cloning step. With this approach, we demonstrate successful targeting of a GFP insertion as well as several endogenous genes at high frequencies. Furthermore, we discuss our CRISPR-based strategies to introduce DNA into the genome by knock-in or homologous recombination, in order to generate functional fusion proteins.

1. Sander JD, Joung JK: **CRISPR-Cas systems for editing, regulating and targeting genomes.** *Nat Biotechnol* 2014.

**OP-SSIII-07**

**Characterisation of insect derived peptidases for the Food Biotechnology***

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The beetles *Tenebrio molitor*, *Rhizopertha dominica*, *Oryzaephilus surinamensis*, *Sitophilus granarius*, *Alphitobius diaperinus* and *Tribolium castaneum* are well known cereal pests. Due to special enzymatic systems possessed by themselves or by symbiotic microorganisms, the beetles are able to use various organic substrates as a food source and therefore, they often cause massive damages. On the other hand, their biochemical potential may be employed for food biotechnology, e.g. for the peptidolytic digestion of gluten.

Peptidase activities of enzyme extracts from the above mentioned beetles were determined. Additionally, their potential to hydrolyse casein and gluten was tested in zymograms. Peptidases derived from the beetles *T. molitor*, *R. dominica*, and *O. surinamensis* showed an efficient hydrolysis of gluten and casein. To localise the source of the peptidase activities, the beetles were deveined, and the activities of the complete and the deveined beetles were compared. Since the peptidase activity in the complete beetle was significantly higher than in the deveined beetle, a localisation of the peptidases in the gastro-intestinal system of the beetles may be concluded.

After purification by FPLC, a serine peptidase from *R. dominica* was identified. The characterisation of this peptidase was performed on a molecular level, the transcriptome of *R. dominica* was sequenced and analysed by bioinformatic tools. The serine peptidase showed activity in zymograms containing gluten, and a hydrolysis of allergy relevant gliadin epitopes was observed.
**OP-SSIII-08**

**Composition and localization of bacterial symbionts in whitefly species (Hemiptera: Aleyrodidae) from the South-East Europe**

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Whiteflies, *Bemisia tabaci* (Gennadius), *Trialeurodes vaporariorum* (Westwood) and *Siphoninus phillyreae* (Haliday) are phloem-feeding pests that cause serious problems in numerous agricultural crops. The world’s two most widespread members of the *B. tabaci* species complex are the Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED). MEAM1 and MED became global invaders and the most damaging, due to the ornamental plants trade. All whitefly species harbour a primary bacterial symbiont and a diverse array of secondary symbionts (SS) which may influence several aspects of the insect’s biology. Molecular markers were used in order to determine the species of *B. tabaci* and to test the presence of SS, while the fluorescent *in situ* hybridization was used to determine the localization of the SS within the insect. Both the MEAM1 and MED *B. tabaci* species were detected in Montenegro, whereas only the MED species was confirmed in Croatia, Bosnia and Herzegovina and Macedonia. *T. vaporariorum* was found to be the predominant species across surveyed areas, while *S. phillyreae* appeared sporadically in the pomegranate growing region. Multiple infections with SS were common in all three whitefly species, however, not all populations harboured all of the tested SS, whereas some of SS showed 100% infection rate in some populations. All SS tested were localized inside the bacteriocyte in all whitefly species, but only *Rickettsia*, *Cardinium* and *Hamiltonella* showed additional localization outside the bacteriocyte. Infections with the same symbionts in reproductively isolated whitefly species confirm complex relationships between whiteflies and bacterial symbionts, and suggest possible horizontal transfer of some of these bacteria.

**OP-SSIII-09**

**Combining host biology with potential applications of insect-associated microbes: the burying beetle example**

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Associations between insects and their symbionts are evolutionarily highly successful. Here we combine the investigation of insect-associated bacteria and fungi with host biology to determine both their impact on the host and their biotechnological potential for enzyme production, compound and biomass transformation. Among the insect-microbe model systems which are being investigated are: i) the clothes moth (*Tineola bisselliella*): a model to study adaptations of insects to a usually inaccessible food source, keratin, ii) the black soldier fly (*Hermetia illucens*): a highly efficient decomposer, and iii) the burying beetle (*Nicrophorus vespilloides*): a necrophore and a model to study social behavior, adaptations to meat conservation and the production of antimicrobials. For the burying beetle we combined RNAseq, GO enrichment analyses of different gut regions and detailed characterization of microbial communities, identifying distinct partitioning in immune, digestive and detoxifying processes along the host gut. Furthermore, while the midgut is essentially a bacteria-free zone, the hindgut is characterized by an abundant and diverse microbiota, including previously unknown yeasts related to the biotechnological model organism *Yarrowia lipolytica*. Based on the metabolic potential of related bacterial and yeast taxa, we hypothesize that the symbionts are involved in detoxification of noxious chemicals and digestion of otherwise inaccessible nitrogen and energy sources. *In vitro* studies with cultured symbiotic yeasts revealed the production of secondary metabolites with antimicrobial properties that have previously been found in anal secretions of the beetles and suggested to play a role in carcass defense.
OP-SSIII-10

Insights into insect-fungus dynamics: a potentially strategic source of novel bioactive compound discovery

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A multitude of insects avoid or readily engage in associations with fungal organisms. Both positive and negative insect-fungus associations are assumed to be mediated by (i) the biosynthesis of fungal specialised metabolites, (ii) by the insects’ ability to perceive such compounds, and/or (iii) to deal with the detrimental/beneficial consequences of fungal chemicals for insect development, physiology and reproduction. In this context, a largely overlooked aspect has been the inducibility of fungal specialised metabolite production in response to insect grazing. I will provide a conceptual overview and first experimental evidence of reciprocally plastic responses in insect-fungus interactions - on the organismic, molecular genetic and biochemical level. In consequences, I hypothesise that in-depth investigations of the ecological and evolutionary dynamics of insect-fungus interactions represent a novel blueprint for yellow biotechnology. This hypothesis is of particular relevance for “awakening” so-called silent fungal specialised metabolite gene clusters that are expressed only under specific ecological conditions, and which may result in the formation of novel antibiotics, chemotherapeutics, or semiochemical biopesticides.

OP-SSIII-11

Proteins and lipids from insect mass cultures: promise and limitations

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The increasing worldwide demand for high quality organic raw material from plants cannot be met without the destruction of valuable ecosystems. In particular soy monocultures and the destruction of rain forests by oil palm plantations pose major ecological problems. The sustainable production of proteins and lipids from insect mass cultures is an attractive option. Larvae of the black soldier fly ($Hermetia illucens$) can be fed with organic waste material and large amounts of biomass can be produced based on secondary resources without competition for agricultural land for human food production. Quantitative and qualitative aspects for the production of organic raw material by insect mass cultures will be addressed as well as the technical and biological problems faced by this approach. In principle, the quality of organic raw material form insects is equivalent to plant material. The lipid composition of the larvae can be varied depending on the quality of the organic substrates used as feed. The energy requirements of the insect mass cultures are of particular concern and need to be addressed to achieve a sustainable production of proteins and lipids. The technological options to separate these major constituents will be discussed. At present, legal constraints limit the large scale use of insects as animal feed and the use of animal proteins and lipids in food products, cosmetics and other consumer products. In addition, acceptance problems hamper the marketing of animal products.
From the oceans to the fields - Producing wax esters in plants

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Wax esters are neutral lipids exhibiting desirable properties for lubrication. Natural sources have traditionally been whales. Additionally some plants, bacteria and insects produce wax esters. Currently there is no biological source available for long chain length monounsaturated wax esters which are most suited for industrial applications. Therefore we are first exploring enzymatic activities from bacteria, insects and plants for the desired properties. Second we analyze their suitability and additional requirements enabling their production in the oil crops Camelina sativa and Crambe abyssinica that we aim to establish as production platforms to produce feed stocks from renewable resources for the chemical industry.

Biocontrol of agricultural pests and mosquitoes

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Drosophila suzukii is one recent invasive pest of small fruits in many parts of the world, whose management is limited to cultural practices and the use of insecticides. We have developed a method to genetically manipulate this species to create strains for the sterile insect technique method of population suppression. This was achieved by the germ-line transformation of D. suzukii with a piggyBac transposon vector having a female-specific lethality effector construct. This can be used in a tetracycline-suppressible conditional gene expression system, when crossed to a suitable tet-transactivator strain. Transformation occurred efficiently, at a frequency of 16 % per fertile G0 embryo injected with vector and helper transposase plasmids. The vector was marked for transformant selection with the polyubiquitin-regulated EGFP fluorescent protein, and contains the attP landing site and heterospecific lox recombination sites for post-integration modification of the transgene vector. The 3xP3-AmCyan fluorescent protein marker was inserted within the lox sites to follow a possible recombinase-mediated cassette exchange, that would allow subsequent improvement of the transgenic strain by immobilisation of the vector and introduction of new marker cassettes. In addition, our groups are working on systems to fight medical relevant vectors like the dengue-transmitting Aedes aegypti mosquito. Fitness evaluations as well as risk-assessment of transgenic insects are main topics of our groups that will also be discussed.
OP-SSIII-14

Modelling inflammatory diseases of the lung in the fruit fly 
*Drosophila melanogaster*

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The incidence of inflammatory diseases of the lung such as asthma or COPD increased dramatically in recent years. Both are typical lifestyle disease meaning that genetic predisposition and environmental factors have to come together to induce pathogenesis. Whereas trigger factors of asthma are predominantly endogenous, those leading to COPD are of external origin. We developed *Drosophila* diseases from both inflammatory diseases that highlight the major phenotypical characteristics of either disease. While ectopic activation of NF-kB and/or FoxO signalling in the airway epithelium induces structural changes that are typically seen in asthma, chronic cigarette smoke exposure reduced life span and induced structural changes similar to those seen in COPD. The detailed molecular analysis of the molecular alterations underlying these disease model revealed a number of relevant signalling systems that might be associated with disease development.

OP-SSIII-15

Development of insect-antenna-based biosensors

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Development of alternative strategies for efficient sterile insect technique mosquito control programs

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Insect vectors are responsible for carrying 20 per cent of all transmittable diseases affecting people in developing countries. Mosquitoes cause the biggest medical and financial burden by spreading malaria, dengue and yellow fever, Japanese encephalitis, Chikungunya virus and West Nile virus. Over the last 30 years, due to increased globalization, population growth, climate change, poverty and poor sanitation, many of these diseases have been re-emerging, with the most rapidly spreading being dengue.

Chemical insecticides remain the most commonly used weapon against the disease. Nevertheless, the establishment of resistance is undermining effectiveness of chemical insecticides for mosquito control. This creates the urgency for developing alternative strategies for vector control. One of the most promising alternatives is the Sterile Insect Technique (SIT). Mass release of sterile male insects has proven a successful strategy for controlling many major insect pests without the disadvantages of using chemical insecticides. Mosquitoes, however, so far resisted this approach because irradiation, the traditional method for sterilizing insects, is detrimental to their viability. In addition, the genetic sterilization approach has also introduced, extensive inbreeding of laboratory lines, genetic manipulation and rearing that compromise mosquitoes’ fitness and mating competitiveness in field applications. Overexpression of proteins participating in fitness improvement and longevity pathways in mosquitoes might be the key for establishing a robust SIT for efficient mosquito control.

From Traditional Maggot Therapy to Modern Biosurgery

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The utilization of fly maggots to the wound healing has been well documented in folk medicine around the world for centuries. Medical maggots, the larvae of the green blow fly, Lucilia sericata, are believed to have three major modes of action on chronic wounds: (i) debridement; (ii) disinfection, (iii) stimulation of healing processes. Although the maggot therapy has been shown to be safe and effective method for the treatment of chronic wounds, it also poses contraindications and adverse effects. Presently several research teams’ worldwide focus on identification and isolation/production of therapeutic substances from medical maggots. The overall tendency is to envisage the switch from traditional maggot therapy to modern biosurgery (to substitute the live animals by potent therapeutic compounds). In our work, we focus on the identification and characterization of maggot derived debridement peptidases. The comprehensive transcriptome database of L. sericata maggots has been prepared and bioinformatically analyzed. Gene specific primers have been designed and the transcription levels of individual genes determined by RT-PCR in different maggot tissues. Chosen enzymatic candidates have been cloned into expression vectors and produced as a His-tagged fusion proteins using Escherichia coli expression system. Recombinant enzymes have been purified, refolded and biochemically characterized.

In addition, we have also investigated the role of maggot excretion products on blood coagulation as a part of the wound healing process. We observed that maggot excretion products strongly induce clotting of human plasma and whole blood and identified the key enzyme(s) responsible for this phenomenon.
**OP-SSIII-18**

**Antimicrobial peptides from the therapeutic maggot *Lucilia sericata***

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The maggots of the green bottle fly *Lucilia sericata* are creepy, crawly, and slimy. But when they were raised under sterile conditions they help to cure open, infected, chronic or necrotic wounds, particularly those whose cure by conventional approaches often fails. The benefits of this widely used maggot therapy include the efficient removal of necrotic tissue (debridement), acceleration of wound healing and wound disinfection. Since the use of living maggots is often distasteful to the patient and the maggots have a short shelf life, a replacement with maggot-derived substances is envisaged. In order to find those substances promoting the healing of a wound we sequenced the transcriptome of *Lucilia sericata*. We found transcripts coding for numerous antimicrobial peptides (AMP) including proline-rich AMPs, defensins with conserved cysteins and α-helical cecropins. 20 of these AMPs were produced by solid phase synthesis and were active against gram positive and gram negative bacteria. One of the predicted peptides could not be assigned to specified AMP classes and was chosen for recombinant production and microbial testing. This 8.2 kDa peptide named Lucimycin is characterized by a high content of histidine and was active against the oomycete *Phytophthora parasitica* and fungi from the phyla Ascomycota, Basidiomycota and Zygomycota, but it was inactive against bacteria.

**OP-SSIV-01**

**Comparative morphology and its indispensable role in phylogenetic systematics**

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Morphology studies the interface between environment and the organism. It therefore traditionally describes the structural correlate of organismic function as response to environmental constraints. Morphology on the other hand is strongly influenced by the evolutionary history of an organism, so that every structural response to environmental factors is a trade-off between environmental and evolutionary constraints. This trade-off results in similar structural solutions as evolutionary response to identical abiotic factors. These are since long recognized as the main cause of convergences and are known as strong burden in attempts to use morphological data for phylogeny inference. Molecular phylogenies are believed not to have to cope with such a problem. Together with an increasingly easier access to sequence data, molecular analyses nowadays seem to be superior to inferences from morphological data. The last comprehensive Hennigian attempt to use morphological data for phylogeny inference was the three volume book by Peter Ax published at the turn of the century. This talk will address the question on the role of comparative morphology in phylogenetics today. Do we really need morphology in the age of next generation sequencing? A closer look at recent publications reveals that despite all progress in morphological techniques knowledge on structural complexity is tremendously decreasing since two decades, along with an increasing belief that morphology is dispensable. This talk will exemplify that such a belief is wrong.
Crustaceans display a particularly high degree of phenotypic disparity. Crustacean taxa differ in tagmatization, limb type, presence or absence of a carapace and in other various aspects. Understanding evolutionary character transformations – which is in the focus of evolutionary biology since 150 years – is therefore a special challenge. In the framework of an Evolutionary Morphology (Richter & Wirkner 2014), an important step is to identify evolutionary units (characters) which are more than just observations but which need to be conceptualized first. As an example the entomostracan “abdomen” will be used, which share the absence of limbs but differ in many other respects. Is the “abdomen” an evolutionary unit (just having evolved once?) or just any combination of limb-less segments? One other goal of Evolutionary Morphology is the identification of ‘coherence’, the non-accidental presence between specific evolutionary units regardless of whether the mechanism responsible is external or internal. This will be exemplified by two case studies, (1) the evolution of cladocerans from a conchostracan-like ancestor, one of the most intriguing cases of evolutionary transformations in crustaceans, and (2) the evolution of a crab-like habitus in several anomuran (Decapoda, Anomala) lineages in an evolutionary process called carcinization. In the former example, the Cyclestherida as sister group to the water-fleas (Cladocera) play a major role because (although a recent taxon) it represents in many respects an intermediate step between clam shrimps and water fleas. In the second case study, we compare carcinization in king crabs (Lithodoidea) with that in porcelain crabs (Porcellanidae). The question we ask is whether or not certain aspects of internal anatomy evolved along with the external shaping into a crab. Because of the obvious convergence of these two cases of carcinization, ‘coherence’ might be better identifiable.

On congruence

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It is widely appreciated that morphological characters are essential in functional analyses of animal tissues and organs, in the interpretation of fossils, and in elucidating evolutionary transformations of morphological features. At the same time, it is often claimed that the phylogenetic analysis of morphological characters contains highly subjective elements and that mapping morphological characters on molecular phylogenies explains character evolution in a more objective fashion. Here I argue that the procedure of mapping is not utilizing the information content inherent in morphological data to their full extent, while simultaneously retaining the supposedly subjective element of a morphological analysis. The crucial central step in comparative morphological studies is the suggestion of hypotheses of homology and this step is retained in character mapping. Mapping manufactures congruence between molecular and morphological datasets. However, in the case that mapped characters would be incongruent to the molecular phylogeny, this contradictory evidence is ignored as a potential falsifier of the molecular phylogenetic hypothesis. In other words: relevant information derived from the morphological analysis is dismissed. Moreover, I claim that morphological structures are valuable for phylogenetic analyses because they integrate ontogenetic interactions at different biological levels. Finally, I demonstrate how some problems of morphological analyses have recently been alleviated by modern technology.
Semantic Approaches to Phylogenetics: Dealing with Character Dependency and Using a Set of Ontology-based Semantic Instance Anatomies instead of a Conventional Character Matrix

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Semantic techniques are increasingly applied in the life sciences. Here I will focus on possible applications in phylogenetic analyses. In the first part of my talk I will present a solution to methodological issues concerning coding of dependent characters in phylogenetics. I introduce the distinction of semantic and ontological logical character dependency. On the basis of typical examples I argue that all ontologically dependent characters have a general underlying scheme in common, irrespective of whether they are based on property instantiation, parthood relations or relations of subsumption. This scheme specifies if-then-rules about necessary combinations of character state values between ontologically dependent characters. When implemented in applications for coding character matrices, a large part of the ontologically dependent character states could be coded automatically. This would significantly facilitate the coding procedure, speed up the coding of large matrices, and prevent coding errors. The rules, however, can only be applied if the dependency relations have been identified in a matrix. Documenting these relations adds another layer of information to the conventional two-dimensional structure of phylogenetic character matrices. However, if ontology-based descriptions of the anatomy of particular specimens (=Semantic Instance Anatomies) are used in the phylogenetic analysis, the identification of ontological dependency relations can be automatized, because all relevant information is already contained in the Semantic Instance Anatomies and their accompanying ontologies. In the second part of my talk I will briefly discuss how Semantic Instance Anatomies can replace conventional two-dimensional character matrices in numerical tree inference, thereby avoiding all problems relating to character coding and logical dependencies.

Phylogenetic Analysis of Salpida (Thaliacea, Tunicata, Chordata) based on Morphological Characters

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Salpida is a high-ranking taxon within Tunicata comprising approximately fifty species in twelve genera. All species are exclusively marine and planktonic. Due to their extraordinary rate of growth and their efficient filter feeding they play an important ecological role. Salps are characterized by a strictly metagenetic life cycle consisting of two morphological distinct generations one reproducing sexually the other asexually. We investigated representatives of all twelve genera and compiled a data matrix that included morphological characters derived from the asexual generation (oozooid, solitary form, proles solitaria) and the sexual generation (blastozooid, aggregate form, proles gregata). New characters are documented using scanning electron microscopy and analyzed in a parsimony framework. We contrast phylogenetic hypotheses derived from both generations separately and combined. In addition, we compare each of the resulting phylogenetic hypotheses to recent molecular analyses of Tunicata and Thaliacea.
PP-SSI-01

Position dependent hearing in three species of bushcrickets (Tettigoniidae, Orthoptera)

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A primary task of auditory systems is the localization of a sound source in space. This task can be difficult for animals living in complex habitats, since sound sources have to be detected in azimuth as well as in elevation. Sound source localization in azimuth is usually based on differences of sounds between the bilaterally arranged ears. Localization in elevation is possible by transfer functions at the ear. Bushcrickets provide good model systems for analysis of sound source localization: they have a sophisticated auditory system including a sensory tonotopy and auditory localization is necessary for reproduction. Apart from the general similarities, different species have distinct morphological adaptations in their hearing systems with different physiologies. Here we compare the auditory systems of three species and show that their hearing thresholds are species specific. Furthermore, hearing thresholds depend on the location of a sound source as is shown for different positions in space along the azimuth and with different elevations. The position dependent tuning is differently pronounced between the three species. The largest differences are found in Ancylecha fenestrata which correlates with a rather complex morphology of its ear including cuticular folds covering the anterior tympanal membranes. The position dependent tuning might contribute to sound source localization in three dimensional complex habitats.

PP-SSI-02

Proliferation and plasticity in adult Tribolium castaneum

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With its fully sequenced genome and the susceptibility for reverse genetics based upon RNA interference (RNAi), the red flour beetle Tribolium castaneum is best suited to study development and plasticity of the nervous system. While plasticity can be provided by various mechanisms we focus on ongoing cell proliferation in the adult beetle brain. It is well established that neurogenesis persists in the mushroom bodies (MB) of adult insects, including the red flour beetle T. castaneum where neuroblasts giving birth to MB Kenyon-cells remain active for more than one month after adult eclosion (Zhao et al. 2008. Devel Neurobio, 68: 1487-1502). Using the 5-ethyl-2'-deoxyuridine (EdU) technique in combination with immunohistochemistry against various neuromediators - including e.g. neuropeptides and NO-synthase - and the glia-cell marker reversed-polarity, we labeled the progenies of adult persisting neuroblasts, determined their identity and counted the newborn Kenyon-cells in the first days after adult eclosion.

In several studies it was proposed that newborn neurons of MBs may play a role during olfactory processing and learning. Currently we combine the EdU-staining with olfactory stimulation using different odors like the leaf alcohol cis-3-hexen-1-ol or the beetle’s aggregation pheromone 4,8-dimethyldecanal (DMD), odor deprivation or knockdown of ORCO to investigate whether the rate of MB neurogenesis depends on olfactory input.
Quantitative Analysis of Neuropeptides in the Antennal Lobes of Male Aedes aegypti

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Mosquitoes probably are the most dangerous animals in the world, as they are vectors of a wide range of viral and parasitic diseases. A reasonable target for pest control is the reproductive cycle, which involves blood feeding as well as mating; both complex behaviors depending to a large extent on olfactory cues. Therefore the aim of our studies is to achieve knowledge of the regulation of olfactory guided behavior. Focusing on the primary olfactory processing centers - the paired antennal lobes - we performed semi-quantitative mass spectrometry to reveal changes in concentration within five neuropeptide families (Tachykinin, short neuropeptide F, Allatostatin-A, Neuropeptide-like precursor and SIFamide) in the course of mating. Male Aedes aegypti were mated three days after adult eclosion. We investigated virgin males and males at several points in time after mating. Interestingly, we revealed a postmetamorphic increase in the concentration of all examined peptides. This increase takes place in the first 5 to 7 days following adult eclosion and subsequently reaches a plateau. In the case of sNPF-2 and SIFa the postmetamorphic increase seems to be decelerated after mating, even though 1 hour post mating both peptides exhibit higher concentrations compared to virgin controls. This finding suggests mating induces short-term as well as long-term effects in the olfactory system of male mosquitoes.

Optical calcium imaging of relative odor concentrations in binary odorant mixtures in the Drosophila antennal lobe

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We investigate how neuronal networks process odor representations resulting in either stimulus categorization or discrimination. We use Drosophila to examine the spatio-temporal representations of different monomolecular odors and their binary mixtures in the first olfactory neuropil, the antennal lobe. Flies perceive odors with olfactory sensory neurons (OSNs) that project into the antennal lobe, which consists of anatomically discrete subunits (glomeruli). OSNs expressing the same receptors converge to the same glomeruli where they synapse onto local interneurons (LNs) and projection neurons (PNs). PNs convey olfactory information to higher brain regions. We express the genetically encoded calcium sensor GCaMP3 in both OSNs and PNs in order to monitor the calcium activity patterns evoked by different concentrations of odors. Glomerular activity patterns change with increasing odor concentrations in OSNs. However, PNs showed a higher degree of invariance over a broad concentration range. To further test whether different sensory inputs are processed by the antennal lobe so that they are discriminated or categorized, we varied both the concentration as well as molecular identity of odors by gradually shifting the concentration ratios of binary odorant mixtures. Changing the concentration ratios of the odorants in the mixtures resulted in abrupt transitions between odor representations at the level of PNs.
ANALYSIS OF CONTACT SITES BETWEEN INTRINSIC AND EXTRINSIC MUSHROOM BODY NEURONS IN DROSOPHILA

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Decades of research have led to a thorough description of the insect mushroom body as a neuronal circuit involved in the control of adaptive behavior, e.g., associative learning. In particular, it has been revealed that mushroom body functions rely on the modulation of the mushroom body intrinsic Kenyon cells by biogenic amines, e.g., octopamine, dopamine or serotonin. Learning-induced plasticity has been proposed to be localized to the contact sites between aminergic neurons and Kenyon cells. Therefore, we asked which modulatory neurons interact with anatomically and functionally distinct mushroom body regions, and where exactly within the mushroom body subdivisions it is that they do so.

We have adapted the recently described splitGFP-technique to label contact sites between mushroom body intrinsic Kenyon cells and extrinsic neurons. Using this approach we find spatially and structurally different contact regions between three neuromodulatory type of neurons and Kenyon cells in different subdivision of mushroom body, as well as spatially distinct zones of connectivity between Kenyon cells and aminergic neurons.

Having localized the contact regions between aminergic neurons and Kenyon cells, we next aimed at addressing the physiology at those defined cellular regions. We therefore generated several transgenic flies that can be combined with the splitGFP label to trace back individual extrinsic or intrinsic neurons or to record neuronal activity using optical imaging. These fly strains can be used to combine physiological and fine-scale anatomical studies, e.g. for monitoring odor-induced activity of Kenyon cells and plasticity at defined anatomical contact regions between Kenyon cells and extrinsic neurons.

Is the circadian clock involved in photo-periodic time measurement in northern Drosophila species?

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Diapause is an insect survival strategy to withstand harsh winter conditions which is characterised by low metabolic activity and the arrest of growth /reproduction. The shortening day-length (photo-period) during summer to winter transition serves as primary environmental cue inducing the diapause. A time measurement mechanism is therefore thought to underlie diapause regulation and commonly termed as photoperiodic clock. Circadian rhythm of activity-rest behaviour is modulated by photo-period and therefore, circadian rhythms are believed to serve as the photoperiodic clock. Many Drosophila species inhabiting temperate zone exhibit robust adult diapause which is characterised by reproductive arrest. We studied the diapause and activity-rest rhythm in Drosophila littoralis and Drosophila ezoana under the light/dark (LD) cycles with varying (a) photo-period and (b) period of LD cycle, to test the circadian involvement in diapause incidence in these species. The diapause incidence was found to be correlated with circadian phenotype suggesting the circadian involvement in diapause regulation. However, in studies under LD cycles with 24-hour and non-24 hour period, the diapause was found to be primarily a function of absolute night-length (duration of dark phase of LD cycle), suggesting the absence of circadian involvement. We hope the behavioural analysis will guide the further investigations into the neuronal and genetic basis of diapause.
Anatomical characterization of the clock neuronal network in the brain of selected insects.

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Circadian clocks have evolved to allow organisms to synchronize their metabolism, physiology and behavior with the external environment. Many insects are able to perform very complex tasks, which require an underlying functioning clock (e.g. sun compass orientation in honeybees and ants), or have developed different strategies to anticipate harsh seasons (e.g. photoperiodic control of diapause in firebugs and drosophilid flies).

Unfortunately, so far not much is known about the anatomical organization of the neuronal networks underlying these particular behaviors. The neuroanatomy of the master clock in the brain of insects mediating such complicated behaviors would be the starting point for their functional analysis. Thus, our first aim is to anatomically characterize the circadian clock network of selected insects (\textit{Apis mellifera}, \textit{Camponotus} ants, \textit{Pyrrhocoris apterus} and \textit{Chymonomiza costata}) to gain the necessary information for a further functional characterization of their circadian clock machinery. Here, our first results are presented.
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