

DZG Section Developmental Biology Presents

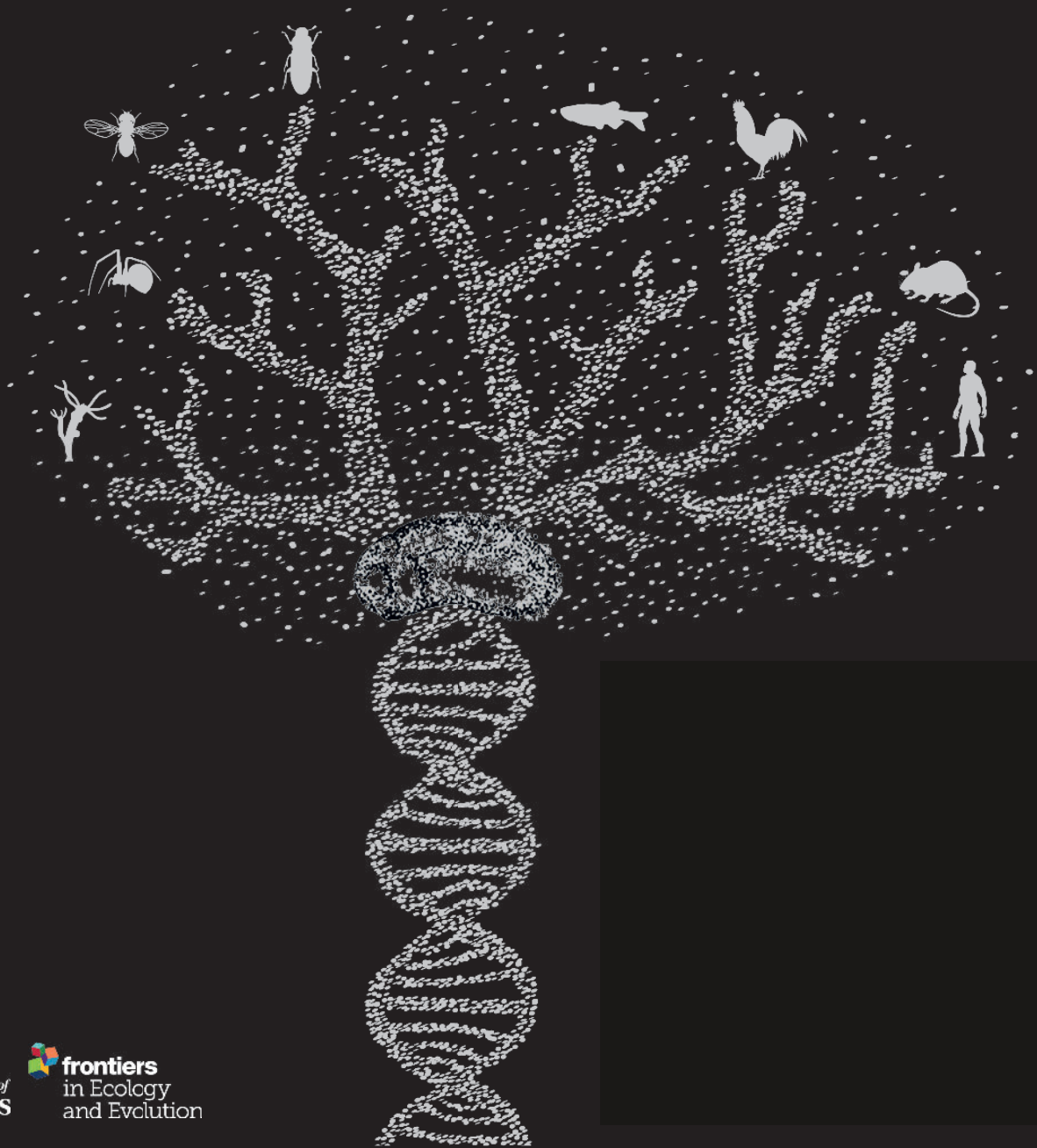
# EVO DEVO

## STATUS QUO

Akademie **WALDSCHLÖSSCHEN** Göttingen

04. - 06. March 2019

Confirmed Speakers **SIEGFRIED ROTH** Köln ·  
**DIETHARD TAUTZ** Plön · **NADIA FRÖBISCH** Berlin ·  
**ANNETTE BECKER** Gießen · **THOMAS BOSCH** Kiel · **MICHAEL HILLER** Dresden ·





## Welcome to “Evo-Devo in Germany: Status quo and future directions”

The animal kingdom is characterized by an impressive diversity of species and morphologies. This diversification is to some extent the result of novel traits, facilitating adaptation to ecological niches and thus resulting in fitness benefits and selective advantages over time. The evolution of this extreme diversity of species and their outstanding variation in morphology and behaviour has fascinated researchers throughout centuries.

Already Ernst Haeckel and Charles Darwin attempted to understand the mechanisms underlying the evolution and development, which led to the establishment of this great diversification.

Advances in genetics and molecular biology provided the ability to study genotype-phenotype relationships in developmental biology in various groups of organisms. Research in evolutionary developmental biology (Evo-Devo) contributed some fundamental concepts in biology. For instance, by comparing the genetic basis of the development of different organs and tissues across closely and distantly related species a high level of conservation in developmental programs has been found. Evo-Devo research also helped to unravel previously unclear phylogenetic relationships.

Fuelled by these advances Evo-Devo researchers incorporated an increasing number of technical tools and theoretical concepts, such as genome editing (CRISPR/Cas9), various computational and mathematical approaches as well as recent sequencing and imaging technologies. Therefore, the Evo-Devo research field has been diverging massively within the last 10 years and is overlapping more and more with other disciplines. These developments are extremely exciting and open new routes to understand the evolution of developmental processes and its consequences on various phenotypes on a mechanistic level. However, we still feel that the communication across disciplines such as morphology, phylogeny, bioinformatics, mathematical modeling, classical developmental genetics, population and quantitative genetics as well as evolutionary theory may profit from more exchange.

Therefore, the main aim of this meeting is to provide a basis for this exchange by bringing together young academics (master and graduate students) interested in Evo-Devo, with long established pioneers of the classical Evo-Devo research field and speakers from other disciplines. We are happy to welcome colleagues working on animal as well as on plants; experimentalists as well as computational biologists and with that cover a variety of approaches to Evo-Devo. We hope that you all enjoy the opportunity for intense discussion and scientific exchange about the state of the art and future of Evo-Devo research in Germany. We also want to encourage young scientists to think outside the box and become active in shaping future directions and collaborations in the broad sense of Evo-Devo.

And now enjoy the meeting and let's discuss.



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Nico Posnien



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Natascha Zhang (aka Turetzek)

# Program

## Monday, March 4, 2019

16:15 - 16:30	arrival at Waldschlösschen/distribute rooms
<b>16:30</b>	<b>Welcome</b>
<b>16:40</b>	<b>Siegfried Roth</b> <i>The evolution of dorsoventral patterning in insects</i>
<b>17:20</b>	<b>Max Farnworth</b> <i>Brain Evo-Devo: Comparing the Tribolium and Drosophila Central Complex</i>
<b>17:40</b>	<b>Felix Kaufholz</b> <i>Is the break-down of posterior segmentation irreversible?</i>
<b>18:00</b>	<b>Gregor Bucher</b> <i>Double abdomen in a short germ insect: Zygotic control of axis formation revealed in the beetle Tribolium castaneum</i>
18:20	Distribute rooms/prepare for dinner
18:30	Dinner
19:45	Speed Dating

## Tuesday, March 5, 2019

8:00	Breakfast
<b>9:00</b>	<b>Diethard Tautz</b> <i>Why complex trait genetics matters for Evo-Devo</i>
<b>9:40</b>	<b>Elisa Buchberger</b> <i>Evolution of the gene regulatory network underlying eye and head development in closely related Drosophila species</i>
<b>10:00</b>	<b>Claudius Kratochwil</b> <i>How cichlid fishes lost and gained their stripes, again and again</i>
<b>10:20</b>	<b>OPEN DISCUSSION 1</b>
11:00	Coffee Break
<b>11:30</b>	<b>Nadia Fröbisch</b> <i>Fossils, bones and genes – the evolution of limb development and regeneration</i>
<b>12:10</b>	<b>Benjamin Naumann</b> <i>Cell organelle composition and chromatin architecture in solitary and colonial choanoflagellates and sponge choanocytes</i>
<b>12:30</b>	<b>Felix Quade</b> <i>Development and metamorphosis in the male pedipalp of the cob-web spider Parasteatoda tepidariorum</i>
13:00	Lunch
13:40	Hiking Trip
15:30	Coffee Break

## Tuesday, March 5, 2019

- 16:00** **Natascha Turetzek**  
*Emerging vs. Main model organisms? Micro- vs. Macro-Evo-Devo? Why choose?*
- 16:20** **OPEN DISCUSSION 2**
- 17:00** **Ting-Hsuan Lu**  
*The identification of the molecular basis underlying eye size differences between *Drosophila melanogaster* and *D. mauritiana**
- 17:20** **Annette Becker**  
*The evolution of land plant reproductive development in a nutshell*
- 18:00** Dinner
- 19:45** Chalk-Talks and Social Evening

## Wednesday, March 6, 2019

- 8:00** Breakfast
- 9:00** **Thomas Bosch**  
*Insights into animal form and function from a basal metazoan perspective*
- 9:40** **Luxi Chen**  
*A cellular study of the unusual diapausing development in *Daphnia* resting embryos*
- 10:00** **Katharina Bachem**  
*A single enhancer tunes pigmentation gene expression to different shades of grey among *Drosophila* species*
- 10:20** Coffee Break/Clear rooms
- 11:00** **Michael Hiller**  
*The importance of cis-regulatory divergence for morphological evolution*
- 11:40** **Nico Posnien**  
*Context dependent regulatory divergence in closely related *Drosophila* species*
- 12:00** **Ralf Schnabel**  
*Cell focussing a basis for morphogenesis?*
- 12:20** Conclusions/discussion/future perspective
- 13:00** Lunch
- 14:00** Departure

## Invited Speakers

### Annette Becker

Justus-Liebig-Universität Gießen, Institute of Botany, Developmental Biology of plants,  
Annette.Becker@bot1.bio.uni-giessen.de

#### ***The evolution of land plant reproductive development in a nutshell***

Life on earth is impossible without plants and during land plant evolution. One of the main challenge plants face on land as compared to (their “previous”) life in the water is their exposure to increased light intensity and water scarcity. Charophyceae algae, the sister lineage to all land plants are aquatic organisms that can rely on water for their sexual reproduction as their flagellate sperm cells swim to the egg cells. But the evolution of sexual reproductive organs in land plants is geared towards independency of water. Sperm cells lost their flagellae, became airborne or took rides on animals, and the fertilization process was, step-by-step, internalized to protect the reproductive organs from dehydration and other detrimental conditions. This trend culminated in angiosperms (or flowering plants) which evolved the carpel that protectively surrounds the ovules (containing female gametes). In these plants, pollen tubes (carrying the male gametes) grow through maternal tissue, guided by chemical signals, allowing fertilization.

Flowering plants are the largest group of plants including over 250.000 species, with the carpel being their most important autapomorphy. My group is interested in identifying the genetic toolkit that was the prerequisite for carpel origin. We use multiple approaches to identify and characterize this presumably highly conserved carpel gene regulatory network and I will present our recent findings.

### Thomas Bosch

Kiel University, Zoological Institute, tbosch@zoologie.uni-kiel.de

#### ***Insights into animal form and function from a basal metazoan perspective***

Basal metazoans such as anemones, corals, and hydras, are seemingly simple animals which diversified from their protistan ancestors 700-800 million years ago, some three billion years after bacterial life originated and as much as a billion years after the first appearance of eukaryotic cells. The genomes of basal metazoans are surprisingly complex, with a gene repertoire, exon-intron structure, and large-scale gene linkage more similar to vertebrates than to flies or nematodes, implying that the genome of the eumetazoan ancestor was similarly complex; and that basal metazoans have retained many genes that have been lost in *Drosophila* and *C. elegans*. High-throughput gene sequencing has uncovered a world of complex interactions between developing organisms and the biotic and abiotic components of their environments where microbes are an essential part of the animal phenotype influencing fitness and thus ecologically-important traits of their hosts. This newfound awareness of the dependency of phenotypes on other species and environmental conditions has re-ignited interest in basal metazoans. Here I highlight the role that basal metazoans have in ecological evolutionary developmental biology. Using the example of Hydra, I address three crucial questions: (i) How do animal hosts communicate with commensal microbes, and how do microbes affect the host phenotype? (ii) Which role do taxonomically restricted genes play in generating evolutionary novelties? And (iii), how do animals control complex behavior and reflexes in their specific environment in the absence of a central nervous system? The comparative analysis of basal metazoan and bilaterian developmental mechanisms may allow the identification of ancient and therefore fundamental principles of animal form and function.

## [Nadia Fröbisch](#)

Natural History Museum Leibniz, Institut für Evolutions- und Biodiversitätsforschung,  
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### ***Fossils, bones and genes – the evolution of limb development and regeneration***

When it comes to the evolution and development of the vertebrate body plan, the limb is one of the best examples of how data from different fields, including morphology, paleontology and developmental biology, have come together to gain a broad understanding of the complex connections between the development and evolution of an organ. Both fossils and genes have provided important insights into the initial evolution of the limb of tetrapods from finned-ancestors and the subsequent diversification of tetrapods.

Despite the great diversity in the form and function of tetrapod limbs, their skeletal development follows a very conservative sequence with a so-called postaxial dominance. Salamanders are the only living tetrapods that deviate from this pattern by showing a reversed, pre-axial polarity in limb development and are also the only tetrapods capable of full limb regeneration. Both features were assumed to be highly derived for salamanders, while it remained largely unknown how preaxial polarity is established developmentally, when it evolved in salamander evolution, and if and how it may be connected to the regenerative capacities of the limbs. New developmental data shows expression patterns of genes with well-known roles in limb development seem to be canonical in early stages of salamander limb development, but very different from other tetrapods in late stages of limb development. Interestingly, data from fossil amphibians and lungfish indicates that both preaxial polarity in limb development and salamander-like regenerative capacities are not derived for modern salamanders, but are much more ancient features that may even be plesiomorphic for all tetrapods.

## [Michael Hiller](#)

Max Planck Institute of Molecular Cell Biology and Genetics and the Max Planck Institute for the Physics of Complex Systems, Dresden, hiller@mpi-cbg.de

### ***The importance of cis-regulatory divergence for morphological evolution***

Morphology is established during development and evolutionary theory predicts that morphology largely evolves by changes in *cis*-regulatory elements. I will present our results on investigating the fate of *cis*-regulatory elements in lineages that lost complex phenotypes: snakes that lost limbs, and subterranean mammals that have degenerated eyes. By combining genome sequencing and functional genomics with genome-wide comparative analyses, we identified thousands of non-coding genomic regions that exhibit higher sequence divergence in snakes and in subterranean mammals, respectively. These diverged genomic regions are significantly associated with genes having key roles in limb and eye development and overlap regulatory elements that are active during normal limb and eye development in related species. Sequence divergence resulted in an extensive loss of relevant transcription factor binding sites, consistent with functional decay of limb and eye regulatory elements in snakes and subterranean mammals. Overall, our analyses provide the first evidence that genome-wide decay of the phenotype-specific *cis*-regulatory landscape is a recurrent evolutionary principle associated with the loss of morphological traits. In the second part, I will introduce a new genomics approach, REforge, that extends the Forward Genomics framework with the goal of improved detection of functional differences in regulatory elements. To this end, REforge measures transcription factor binding site divergence instead of sequence divergence for all branches in a phylogenetic tree. We show that this approach substantially improves our ability to detect regulatory elements, whose divergence is associated with morphological differences between species.

Combining comparative and functional genomics represents a general strategy that has great potential to reveal the genomic basis of morphological changes, which will help to unravel the mechanisms underlying evolutionary differences in development.



## Siegfried Roth

University Cologne, Zoological Institute, Biocenter, Department of Developmental Biology,  
siegfried.roth@uni-koeln.de

### ***The evolution of dorsoventral patterning in insects***

Toll-dependent patterning of the dorsoventral axis in *Drosophila* represents one of the best-understood gene regulatory networks. However, its evolutionary origin has remained elusive. Outside the insects Toll is not known for a patterning function, but rather for a role in pathogen defense. I will present our recent findings on the evolution of dorsoventral patterning in insects. In particular, I will describe our work on a hemimetabolous insect, the milkweed bug *Oncopeltus fasciatus*, whose lineage split from *Drosophila*'s more than 350 million years ago. In *Oncopeltus*, Toll is only required to polarize a dynamic BMP signaling network. Modeling of this network reveals that shallow Toll signaling gradients are sufficient to initiate axis formation. Dynamic BMP signaling combined with long-range, shallow Toll signaling gradients can explain the twinning of embryos upon egg fragmentation, which has been observed in many insect lineages.

## Diethard Tautz

Max Planck Institute for Evolutionary Biology Plön, Evolutionary Genetics, tautz@evolbio.mpg.de

### ***Why complex trait genetics matters for Evo-Devo***

The new insights coming from complex trait genetics are about to revolutionize our understanding of how biological systems work. This affects all fields of biology, but is also of special interests for Evo-Devo research. It provides new experimental entry points into studying the genetic mechanisms of morphological adaptations. It provides also possible solutions for the old question of macroevolution versus microevolution. Much of our work focuses on understanding who skeletal elements (skull and limbs) can evolve and I will discuss our approaches and findings.



## Contributed Talks

### Katharina Bachem

LMU Munich, Evolutionary Ecology, AG Gompel, katharina.bachem@campus.lmu.de

#### ***A single enhancer tunes pigmentation gene expression to different shades of grey among Drosophila species***

The diversification of morphological traits entails changes in size, changes in shape, as well as extensive quantitative variation of other dimensions of the traits. How gene regulatory networks controlling the formation of morphological traits during development accommodate this quantitative variation during evolution is not understood. Here, to address this problem, we examine quantitative changes in pigmentation intensity of a wing pattern element among closely related species of *Drosophila* fruit flies. We show that the variation, ranging from light grey to intense black in adult flies, correlates with quantitative variation in the developmental expression of yellow, a gene necessary for the formation of black pigments. We found that a single enhancer of yellow, at the origin of its expression in the wing, has changed its activity levels between species, determining the extent of darkness on the wing. Our results suggest that quantitative variation in trait properties that fuels morphological diversification may primarily evolve through the regulatory modulation of the effector genes that produce these traits.

### Elisa Buchberger

Georg-August-University Göttingen, Johann-Friedrich-Blumenbach Institute for Zoology and Anthropology, Department of Developmental Biology, ebuchbe@gwdg.de

#### ***Evolution of the gene regulatory network underlying eye and head development in closely related Drosophila species.***

Insect compound eyes are highly complex organs, which are composed of individual subunits, so called ommatidia. We have recently shown that closely related *Drosophila* species show remarkable differences in eye size and head shape. The eye size differences between *D. melanogaster* and *D. mauritiana* are a result of differences in the number of ommatidia. We use this model to identify the molecular changes underlying the observed morphological variation in adult structures and try to understand how gene regulatory networks (GRN) in closely related species evolve. A comparative developmental transcriptome dataset combined with a transcription factor binding site analysis showed that the GATA factor Pannier (Pnr) regulates many genes that are differentially expressed between *D. melanogaster* and *D. mauritiana*. We found that the transcript of *pnr* itself is differentially expressed in the two species during eye development. Additionally, we could show that *u-shaped* (*ush*), coding for a co-factor of Pnr, is transcribed and translated in the developing eye-antennal disc. We used the binary GAL4-UAS system and subsequent antibody staining to reveal that the two factors regulate each other. To test, if the regulatory module composed of Pnr and Ush may represent a flexible node in the eye and head developmental GRN, we overexpressed *pnr* and *ush*, respectively in the eye-antennal disc in *D. melanogaster*. We indeed were able to phenocopy aspects of the differences observed between the *D. melanogaster* and *D. mauritiana*, showing that higher levels of Pnr lead to a bigger eye area and a narrower, interstitial face cuticle. In summary, our data suggests that differences in the expression of *pnr* and *ush* might explain part of the variation observed between the head shapes of *D. melanogaster* and *D. mauritiana*.

## Gregor Bucher

Georg-August-University Göttingen, Johann-Friedrich-Blumenbach Institute for Zoology and Anthropology, Department Evolutionary Developmental Genetics, gbucher1@uni-goettingen.de

### **Double abdomen in a short germ insect: Zygotic control of axis formation revealed in the beetle *Tribolium castaneum***

The distinction of anterior versus posterior is a crucial first step in animal embryogenesis. In the fly *Drosophila*, this axis is established by morphogenetic gradients contributed by the mother. This strictly maternal contribution regulates zygotic target genes. This principle has been considered to hold true for insects in general but is fundamentally different from vertebrates where zygotic genes and Wnt signaling are required.

We investigated symmetry breaking in the beetle *Tribolium castaneum*, which among insects represents the more ancestral short germ embryogenesis. In order to identify novel components, we mined the data gathered by the genome wide RNAi screen iBeetle. We found that maternal *Tc-germ cell-less* is required for anterior localization of maternal *Tc-axin*, which represses Wnt signaling and promotes expression of anterior zygotic genes. Both, RNAi targeting *Tc-germ cell-less* or double RNAi knocking down the zygotic genes *Tc-homeobrain* and *Tc-zen1* led to the formation of a second growth zone at the anterior, which resulted in double abdomen phenotypes. Conversely, interfering with two posterior factors, *Tc-caudal* and Wnt, caused double anterior phenotypes.

These findings reveal that not only maternal but also zygotic mechanisms including Wnt signaling are required for establishing embryo polarity and induce the segmentation clock in a short germ insect.

## Georg Bullinger

Georg-August-University Göttingen, Johann-Friedrich-Blumenbach Institute for Zoology and Anthropology, Department of Developmental Biology, European Neuroscience Institute Göttingen, g.bullinger@stud.uni-goettingen.de

### **Evolution of visual properties in closely related *Drosophila* species**

The closely related species *Drosophila melanogaster*, *D. simulans* and *D. mauritiana* have different eye sizes. This results from a difference in ommatidia number and size. Rhodopsin 3 (rh3) expressing ommatidia have a larger diameter and *D. mauritiana* shows the highest rh3 expression and the largest diameter of ommatidia. To investigate this correlation further, eyes stained with rh3-antibodies are morphologically analysed. Behavioural experiments are being performed to find potential differences in visuomotor responses between these species. Furthermore, fly lines are being generated to utilise the Gal4-UAS system for calcium imaging in laminar monopolar neurons in these species.

## [Luxi Chen](#)

Ruhr-Universität Bochum, Lehrstuhl für Evolutionsökologie und Biodiversität der Tiere,  
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### ***A cellular study of the unusual diapausing development in Daphnia resting embryos***

Diapause is a form of dormancy, predetermined by the genotype allowing animals to overcome harsh environmental conditions. During the phase of apparent death, development, growth and metabolic activity are depressed until distinctive environmental cues signal favorable living conditions. Metabolic depression during diapause is quite challenging for cells, as they must maintain their viability at reduced energy flows. However, the mechanisms that control halted development during diapause have not been thoroughly described. We investigated diapause related changes on the cellular level in diapausing and non-diapausing *Daphnia magna* embryos. Using (immuno-)fluorescent labeling, we observed the expressions of cell cycle associated proteins, nucleolar proteins, cytoskeletal proteins, and histone proteins. We also quantified the expression patterns of associated genes with the help of qPCR. We found that, the cytoskeleton is gradually reduced to a minimum, rendering diapausing cells compact and condensed. Accompanied by a downregulation of the proliferating cell nuclear antigen (PCNA), the mitotic activity is brought to a halt during diapause. We speculate that the expressions of modified histone proteins points to a decline in DNA transcription during diapause. At the same time, we observed that the diapausing cells still maintain their nucleoli, which may indicate ongoing RNA translation. In this context we found high levels of 18S mRNA in diapausing cells. Our results suggest that cells in diapause have evolved some unique strategies that allow long-term suspended animation maintaining the capacity of resurrection.

## [Max Farnworth](#)

Georg-August-University Göttingen, Johann-Friedrich-Blumenbach Institute for Zoology and Anthropology, Department Evolutionary Developmental Genetics, mfarwo@gwdg.de

### ***Brain Evo-Devo: Comparing the Tribolium and Drosophila Central Complex***

The central complex is an insect brain neuropil which acts as information processing module, receiving mostly visual input and facilitating the resulting motobehavioural output. Its structure is highly conserved in adult insects, but astonishingly the developmental timing differs greatly between species. For instance, in *Schistocerca* the entire central complex forms during embryogenesis while in *Drosophila* it develops postembryonically. *Tribolium* takes an intermediate position where the central complex forms partially during embryogenesis and is completed postembryonically.

My work focuses on this heterochronic shift during central complex development. I am attempting to compare central complex development of *Drosophila melanogaster* to that of *Tribolium castaneum* to identify the cellular basis of these developmental differences. I ultimately aim to contribute to the understanding by which cellular mechanisms a conserved adult structure can show vastly different developmental schemata.

A meaningful comparison requires the marking of homologous cells throughout development. This is achieved by marking cells in both species using the expression of the highly conserved transcription factor Retinal Homeobox (Rx). Rx is expressed in cells contributing to the central complex of both species and after knockdown a central complex phenotype is found in *Drosophila* as well as in *Tribolium*. First potential homology of a sub-group of Rx-positive cells will be established in the adult brain of *Tribolium* and *Drosophila* by antibody staining and transgenic lines. The development of the marked cells will then be followed back from the adult to the embryo where Rx expression starts. The detected differences will be correlated with neuroblast numbers and types, apoptosis, proliferation and cell division. This will hopefully provide insights into the cellular mechanisms underlying the heterochronic shift as well as its exact timing. We propose that the genetic marking of homologous cells and their comparison between species promises to aid uncovering the cellular and developmental basis of brain evolution.

## Muhammad Salim Hakeemi

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### **Tools to study zygotic gene functions in *T. castaneum***

A major strength of the *T. castaneum* model system is its efficient systemic parental RNAi response. However, sometimes the systemic nature of RNAi response limits the gene function analyses in *T. castaneum* due to maternal sterility and lethality. For instance, if a gene has an important role during oogenesis, the injected females will become sterile and the knockdown phenotype cannot be studied in the offspring (*Tc-wg*, *Tc-dpp* and *Tc-cactus*). Additionally, if the gene function is required at different stages of embryogenesis then the resulting knockdown phenotype will be the mixture of all gene functions. Therefore, to overcome the problems of sterility and lethality after parental RNAi, we are generating viral suppressor of RNAi (VSR) lines. The generation of VSR lines will facilitate to study zygotic gene functions and will open new experimental possibilities in emerging model organism *T. castaneum*.

## Felix Kaufholz

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### **Is the break-down of posterior segmentation irreversible?**

The red-flour beetle, *Tribolium castaneum*, is a classic example of an insect with short-germ development. In contrast to long germ segmentation (like employed by *Drosophila melanogaster*), where all segments are formed more or less simultaneously during the blastoderm stage, in the short germ mode only certain anterior segments are patterned during the blastoderm while more posterior segments are progressively added during germ-band elongation in a segment addition zone (SAZ). In *Tribolium*, head and thorax are patterned during the blastoderm, while abdominal segments are patterned during the germ band stage. The SAZ in *Tribolium* is set up before and during the blastoderm stage by *torso* and Wnt signaling and maintained through germband extension by *caudal* (*cad*). In this SAZ, the three primary pair-rule genes (pPRG) *even-skipped*, *run*, and *odd-skipped* (*eve*, *run*, *odd*) are theorized to oscillate during both blastoderm and germband extension stages, resulting in a clock-and-wavefront patterning mechanism. Although some general functions of involved genes were studied in the short germ band mode of segmentation, the details of the gene regulatory network, especially the interactions/hierarchy of the genes with each other is not fully understood. I performed a gene knockdown screen via RNAi for known segmentation genes that result in body axis truncations, in combination with heat shock mediated RNAi rescue at different time points during segmentation using a novel transgenic tool, hsVSR (heat shock viral suppressor of RNAi). A rescue of abdominal segmentation can be observed for *eve*, but not for the other two pPRGs. RNAi against a secondary pair-rule gene can also be rescued. No rescue of abdominal segmentation was observed for RNAi of Wnt-pathway components. This data suggests that the breakdown of the SAZ itself is irreversible while the oscillator can be restarted, at least in the case of *eve* RNAi. The fact that only one out of three pPRGs can be rescued is surprising. Assuming the pPRGs are oscillating during germ-band elongation, why can only *eve* be rescued? Are there biological or technical reasons for our inability to rescue *run* or *odd*? We will continue to characterize the rescue of *eve*<sup>RNAi</sup> by analyzing the expression of the pPRGs, *cad*, and *wg* in RNAi and rescue germ bands. This will further include qPCR expression analysis of *eve*, *run*, and *odd*. We also plan to extend the characterization to *run*<sup>RNAi</sup> and *odd*<sup>RNAi</sup>. At this point it remains unclear why *eve*, but not the other two pPRGs are rescuable, with possible wide-reaching implications for the current model of *Tribolium* segmentation.

## Claudius Kratochwil

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### ***How cichlid fishes lost and gained their stripes, again and again.***

Cichlid fishes are a striking example for repeated 'explosive' evolution forming some of the largest species radiations among vertebrates. In less than ten million years over 1,200 species evolved alone in the species flocks of the three large East African Lakes Victoria, Tanganyika, and Malawi. This astonishing biodiversity with a wide variety of color patterns makes them an ideal vertebrate system to investigate the developmental and genomic changes underlying color pattern diversification and convergence, the evolution of similarities. Coloration is undoubtedly one of the traits that strongly affected the diversification and exceptional rates of speciation we find in cichlid fishes. Understanding the molecular basis of color patterns might therefore help us to understand the mysteries behind the species richness and diversity of cichlids. Horizontal stripe patterns are a particularly striking example of repeated evolution as they evolved and got lost dozens of times within African cichlids. Interestingly, we find that although stripes evolved independently in different species and even in different African Lakes, their losses and gains are triggered by independent mutations of a single gene, *agrp2*. In fact, we show that the inactivation of *agrp2* using Crispr-Cas genome engineering can reconstitute stripes in a normally non-striped cichlid fish. Our results therefore demonstrate how different changes of a single gene drove the repeated evolution of a color pattern across the species-rich African cichlids.

## Ting-Hsuan Lu

Georg-August-University Göttingen, Johann-Friedrich-Blumenbach Institute for Zoology and Anthropology, Department of Developmental Biology, tlu@gwdg.de

### ***The identification of the molecular basis underlying eye size differences between *Drosophila melanogaster* and *D. mauritiana*.***

The size and shape of adult organs is controlled by developmental gene products, which are organized in gene regulatory networks (GRNs). To reveal the molecular basis of natural variation in complex trait morphology, we study the evolution of compound eye size and head shape in different *Drosophila* species. As previous studies have shown, the size of the head cuticle and the eyes are negatively correlated in various *Drosophila* species. Compared to *D. melanogaster*, *D. mauritiana* has larger eyes which form at the expense of head cuticle. Ongoing work has shown that the GATA family transcription factor Pannier (*Pnr*) represents a flexible node in the GRNs.

However, genetic tests suggest that the causative genomic changes lie upstream of the *pnr* locus. We combine various datasets to identify putative evolving regulators of *pnr*: 1) Based on a genome wide expression dataset in combination with transcription factor motif analyses, we identified several candidates that bind to regulatory elements of *pnr* (e.g. *Mad*, *Jim*, *Zen*). 2) Previously published quantitative genetics datasets aiming at revealing candidate changes responsible for intra-specific head cuticle variation revealed genes that may be involved in the observed interspecific variation (e.g. *jim*, *Fasciclin 3*, *Reticulon-like1*). 3) We use allele-specific expression data to identify genes that show signatures of cis-regulatory divergence (e.g. *zeste*, *trf*, *cort*). To test which of these candidate genes is indeed responsible for morphological differences between *D. melanogaster* and *D. mauritiana* we combine reciprocal hemizyosity tests based on CRISPR/Cas9 mutagenesis with developmental genetics approaches. Eventually, we aim at unveiling the role of the identified evolving gene(s) in the GRN underlying head formation in *Drosophila*.

## Benjamin Naumann

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### **Cell organelle composition and chromatin architecture in solitary and colonial choanoflagellates and sponge choanocytes**

Choanoflagellates are the closest relatives to the multicellular animals, the Metazoa. Despite first described as single-celled organisms, some choanoflagellates have the ability to form colonies consisting of more than 100 cells. In contrast to metazoans, these choanoflagellate colonies have been described to consist of similar cells that show no sign of differentiation into different cell types. Therefore, cell differentiation and the presence of different cell types is used as one of the basic features to define the Metazoa. Recently, the discovery of single cells in colonies of the choanoflagellate *Salpingoeca rosetta* that exhibit a unique cell form challenges this view. We therefore asked: How similar are the cells within a colony really compared to solitary *S. rosetta* and sponge choanocytes? To answer this question, we used high-resolution TEM serial sections through three *S. rosetta* colonies (7, 10 and 12 cells), three solitary cells and five choanocytes of the sponge *Oscarella carmela* to reconstruct the organelle composition (food vacuoles, mitochondria, nuclei, etc) of each cell. Additionally, we reconstructed the microscopic euchromatin/heterochromatin composition in each nucleus to compare the diversity of chromatin ratios and architecture of differentiated sponge choanocytes to “undifferentiated” *S. rosetta* cells.

## Nico Posnien

Georg-August-University Göttingen, Johann-Friedrich-Blumenbach Institute for Zoology and Anthropology, Department of Developmental Biology, nposnie@gwdg.de

### **Context dependent regulatory divergence in closely related *Drosophila* species**

The great morphological diversity that can be observed in different animals is the result of millions of years of evolution of the underlying developmental programs. Developmental gene regulatory networks (GRNs) need to be highly constraint to ensure consistent organ formation throughout varying environmental conditions. However, these networks also must be flexible enough to allow natural variation in organ morphology to occur.

Since many developmental genes are highly conserved across distant animal phyla, it has been proposed that gene expression divergence plays an important role in phenotypic diversification.

Here, we study tissue and stage specific regulatory divergence in developing wings and heads between the three closely related *Drosophila* species *D. melanogaster*, *D. simulans* and *D. mauritiana*. We analyzed genome wide expression differences between species and compare these to allele specific expression in F<sub>1</sub> hybrids. This way we can infer whether inter-species expression differences are due to changes in the *cis*-regulatory region of a gene or due to changes in upstream factors that regulate their expression (variation in *trans*). Our results indicate that most differences are due to changes in *trans*. A detailed analysis of gene expression in the context of developmental GRN connectivity revealed that a few central and highly connected genes vary in expression, what explains the extent of *trans*-regulatory differences observed. Additionally, we show that developing wing and head tissue show differences in the regulatory divergence. In summary, our data strongly suggests that gene regulation evolves tissue and stage specifically.



## Felix Quade

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### ***Development and metamorphosis in the male pedipalp of the cob-web spider *Parasteatoda tepidariorum****

Tracing from marine ancestors, spiders have adapted to a terrestrial lifestyle independent from the insects. Many of the adaptations of spiders to life on land involve the shape and function of their appendages. From these appendage modifications, the transformation of the tarsal tip of the male pedipalp into an intromittent organ, the bulbus organ, is particularly interesting. Being an autapomorphy for spiders (i.e. a distinctive feature that is unique to a given taxon) the bulbus' role as intromittent organ for sperm transfer on land accounts for a large part of their evolutionary success. My research on the adult morphology and development of the bulbus organ of the theridiid spider model *Parasteatoda tepidariorum* has answered fundamental questions. It was confirmed that the adult bulbus is built up in a tripartite way, as described by Agnarsson and colleagues (2007). It is built of the subtegulum, tegulum and the embolic section which are connected through membranes, the haematodochae. The embolic section gives rise to the sclerites which are involved in the connection and penetration of the female during the copulatory act. Furthermore, an innervation was found and a sensilla, which appears to be similar to that in the bulbus of *Philodromus cespitum* (Sentenská et al. 2017). The data on the development showed that the bulbus originates in the claw fundament and its primordium is already built in the stage before the penultimate moult. Through a newly discovered mechanism tibia and cymbium are built from scratch which involves coagulation of haemolymph and reorganisation of the coagulated material by cell clusters. Together these findings clearly show that a comprehensive analysis of the morphology over its ontogenesis yields deep insights. However, at the same time new questions arose which now must be answered with future research endeavours.

## Ralf Schnabel

Technische Universität Braunschweig, Developmental Genetics, r.schnabel@tu-bs.de

### ***Cell focussing a basis for morphogenesis?***

Ralf Schnabel, Daniel Findeis and Christian Hennig

*C. elegans* embryos are an ideal system to analyse cell behaviour using 4D-microscopy. Bioinformatical analyses show that pattern formation is achieved by sorting cells—through far-ranging movements—into coherent regions, which are the basis for the morphogenesis of the worm. The sorting of cells is coupled to their particular fate and manipulations of cell fates cause cells to arrange in new patterns, even across the whole embryo. It appears that cells navigate by reading the identities/addresses of their neighbours. Cells are not mobilised by specific signals, but move all the time by dancing. This dance is biased by the addresses/identities of neighbours into their appropriate positions in the body plan. Not the dance itself but its directional bias depends on the SCAR/WAVE complex—discriminating general movement from guided migration. Since we also observe the dance of cells in such diverse systems like *Drosophila*, zebrafish or fibroblasts it appears conceivable that cell focussing may more generally guide morphogenesis in animals. It will be discussed briefly, why all attempts to identify the molecular nature cell address system in *C. elegans* have failed so far. Nevertheless, we propose that an evolutionary modification of the postulated address system may be a very parsimonious strategy to modify archetypical body plans.



## [Natascha Zhang \(Turetzek\)](#)

LMU Munich, Department Biology II, Evolutionary Ecology, zhang@bio.lmu.de

### ***Emerging vs. Main model organisms? Micro- vs. Macro-Evo-Devo? Why choose?***

Perception of environmental stimuli is crucial for the survival of any organism. The diversity of sensory organs is in no way inferior to the great morphological diversity present in the animal kingdom. Over the last decades, the morphology, cellular architecture and developmental origin of various arthropod sense organs has been intensively studied. The genetic basis of variation in sensory organ development, especially detailed gene regulatory networks (GRNs) and their level of conservation, are less well understood. Within arthropods many sensory modalities are perceived through cuticular external sensilla innervated by neurons. In *D. melanogaster* it is known that such sensilla develop from epithelial cells, where proneural cell clusters are determined by the expression proneural genes. Lateral Inhibition via the Notch signaling pathway refines the differentiation into sensory organ precursors (SOP). The fate of the SOP is further influenced by, so-called SOP identity genes. I will study the level of GRN conservation and where alterations in the GRNs occur during development leading to the diversification of external arthropod sensory organs following Macro- and Micro-Evo-Devo approaches. Using comparative candidate gene expression and functional studies in the spider *Parasteatoda tepidariorum* I will identify the level of conservation across arthropods. With single cell sequencing of closely related *Drosophila* species with diversified bristle patterns on the ovipositor I will reveal details of alteration in sensory organ GRNs during development.

# Travel Information

## Arrival

There is a regular bus connection from the main train station to the Akademie Waldschlösschen (see bus schedule below). On Monday, March 4 at 15:50 a delegation from Göttingen will take the bus and it would be great if you'd join us there. We propose to meet at 15:30 at the memorial for the "Göttinger Sieben" in front of the train station.

Of course, you can also get to the Waldschlösschen by Taxi if you prefer.

## 155 Göttingen - Reinhausen - Nesselröden - Duderstadt → 155

Regionalbus Braunschweig GmbH, ☎ (08 00) 5 11 30 02, e-mail: service.regiobusnord@deutschebahn.com

**Am 24. und 31.12. Verkehr wie Samstag! Sind der 24. bzw. 31.12. ein Sonntag, gilt das Fahrplanangebot für Sonn- und Feiertage!**

**Das gesamte Fahrtenangebot zwischen Göttingen und Duderstadt entnehmen Sie bitte dem Fahrplan GF60.**

**Am Tag der Zeugnisausgabe kann es bei einigen Fahrten zu geringfügigen Veränderungen kommen. Bitte informieren Sie sich rechtzeitig.**

**Die Haltestelle "Diemarden Kleebreite" wird bei Straßenglätte nicht bedient!**

Fahrtnummer	Montag bis Freitag																				
	5155 007	5155 401	5155 201	5155 405	5155 403	5155 433	5155 411	5155 211	5155 013	5155 205	5155 415	5155 217	5155 417	5155 419	5155 435	5155 423	5155 025	5155 027	5155 029	5155 031	
Verkehrsbeschränkungen	S	F	S	S	S	S	S	F	F	S	F	S	S	S	S	S	S	S	S	S	S
Anmerkungen	☞		☞																		
Göttingen Bahnhof/ZOB	6:10	6:25								7:30	7:35	9:35	11:35	12:30	12:50						
Göttingen Mänensstraße	6:11	6:26								7:31	7:36	9:36	11:36	12:31	12:51						
Göttingen Bunsenstraße	6:13	6:28								7:33	7:38	9:38	11:38	12:33	12:53						
Göttingen Neues Rathaus	6:14	6:29								7:34	7:39	9:39	11:39	12:34	12:54						
Göttingen Leibnizstr.	6:15	6:30								7:35	7:40	9:40	11:40	12:35	12:55						
Göttingen Gothaer Platz	6:16	6:31								7:36	7:41	9:41	11:41	12:36	12:56						
Göttingen Magdeburger Weg	6:15	6:31								7:36	7:41	9:41	11:41	12:36	12:56						
Göttingen Kiefernweg	6:17	6:32								7:37	7:42	9:42	11:42	12:37	12:57						
Göttingen Hauptstraße	6:18	6:33								7:38	7:43	9:43	11:43	12:38	12:58						
Göttingen Mitteldorfstraße	6:19	6:34								7:39	7:44	9:44	11:44	12:39	12:59						
Göttingen Kurmainzer Weg	6:20	6:35								7:40	7:45	9:45	11:45	12:40	13:00						
Klein Lengden Kirche																					
Klein Lengden Zum Alten Bahnhof																					
Diemarden Kleebreite	6:24	6:39								7:44	7:49	9:49	11:49	12:44	13:04						
Diemarden Schulstraße										7:45	7:50	9:50	11:50	12:45	13:05						
Diemarden Bahnhofstraße	6:26	6:41								7:46	7:51	9:51		12:46							
Diemarden Sportplatz	6:27	6:42								7:47	7:52	9:52		11:52	12:47	13:10					
Reinhausen Knüllstraße	6:29	6:44								7:49	7:54	9:54		11:54	12:49	13:12					
Reinhausen Kirchberg	6:30	6:45								7:50	7:55	9:55		11:55	12:50	13:13					
Reinhausen Reinstraße	6:31	6:46								7:51	7:56	9:56		11:56	12:51	13:14					
Bettenrode Wendebachtal	6:32	6:47								7:52	7:57	9:57		11:57	12:52	13:15					
Waldschlösschen Wendebachtal	6:34	6:49								7:54	7:59	9:59		11:59	12:54	13:17					
Appenrode Wendebachtal	6:35	6:50								7:55	8:00	10:00		12:00	12:55	13:18					

## Departure

Waldschlösschen Wendebachtal	5:19	6:19	6:49	7:11	8:54	10:54	12:54	13:09	13:54	14:17	15:06	16:04	16:06	17:54
Bettenrode Wendebachtal	5:20	6:20	6:50	7:42	8:55	10:55	12:55	13:10	13:55	14:15	15:07	16:05	16:07	17:55
Reinhausen Reinstraße	5:22	6:22	6:52	7:44	8:57	10:57	12:57	13:12	13:57	14:17	15:09	16:07	16:09	17:57
Reinhausen Kirchberg	5:23	6:23	6:53	7:45	8:58	10:58	12:58	13:13	13:58	14:18	15:10	16:08	16:10	17:58
Reinhausen Knüllstraße	5:24	6:24	6:54	7:46	8:59	10:59	12:59	13:14	13:59	14:19	15:11	16:09	16:11	17:59
Diemarden Sportplatz	5:26	6:26	6:56	7:48	9:01	11:01	13:01	13:16	14:01	14:21	15:13	16:11	16:13	18:01
Diemarden Schulstraße				7:50										
Diemarden Bahnhofstraße	5:27	6:27	6:55	7:52	9:02	11:02	13:02	13:17	14:02	14:22	15:14	16:12	16:14	18:02
Diemarden Kleebreite	5:29	6:29	6:57	7:54	9:04	11:04	13:04	13:19	14:04	14:24	15:16	16:14	16:16	18:04
Klein Lengden Zum Alten Bahnhof														
Göttingen Kurmainzer Weg	5:33	6:33	7:01	7:03	9:08	11:08	13:08	13:15	13:23	14:08	14:28	15:20	16:18	16:20
Göttingen Mitteldorfstraße	5:34	6:34	7:02	7:04	9:09	11:09	13:09	13:16	13:24	14:09	14:29	15:21	16:19	16:21
Göttingen Hauptstraße	5:35	6:35	7:03	7:05	9:10	11:10	13:10	13:17	13:25	14:10	14:30	15:22	16:20	16:22
Göttingen Kiefernweg	5:36	6:36	7:04	7:06	9:11	11:11	13:11	13:18	13:26	14:11	14:31	15:23	16:21	16:23
Göttingen Magdeburger Weg	5:36	6:36	7:04	7:06	9:11	11:11	13:11	13:18	13:26	14:11	14:31	15:23	16:21	16:23
Göttingen Gothaer Platz	5:37	6:37	7:05	7:07	9:12	11:12	13:12	13:19	13:27	14:12	14:32	15:24	16:22	16:24
Göttingen Leibnizstr.	5:38	6:38	7:06	7:08	9:13	11:13	13:13	13:20	13:28	14:13	14:33	15:25	16:23	16:25
Göttingen Bürgerstraße	5:40	6:40	7:08	7:10	9:15	11:15	13:15	13:22	13:30	14:15	14:35	15:27	16:25	16:27
Göttingen Angerstraße	5:41	6:41	7:09	7:11	9:16	11:16	13:16	13:23	13:31	14:16	14:36	15:28	16:26	16:28
Göttingen Grüner Tor/Hirtenstr.	5:42	6:42	7:10	7:12	9:17	11:17	13:17	13:24	13:32	14:17	14:37	15:29	16:27	16:29
Göttingen Bahnhof/ZOB	5:46	6:46	7:14	7:16	9:21	11:21	13:21	13:28	13:36	14:21	14:41	15:33	16:31	16:33



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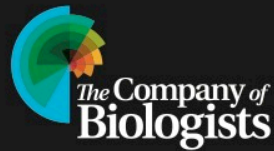
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