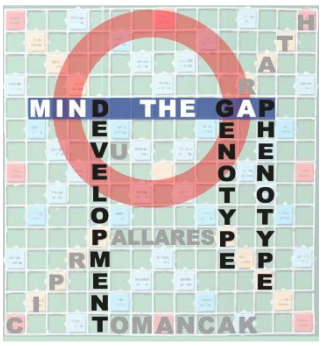
Developmental Biology section of the German Zoological Society (DZG)

Mind the gap: From Genotype to Phenotype and the role of Modelling, Genomic Prediction, and Development



Program

All Sessions will be available via Zoom:

Notepad for discussions:

https://pad.gwdg.de/jdWoFqGfTteMhobhmJdKwA?both

all times CET

invited talks:	35 minutes incl. questions
contributed talks:	16 minutes incl. questions
short talks:	8 minutes incl. questions

Wednesday, March 2, 2022

12:45 **Welcome**

13:00 **Sonja Grath**

Understanding how genetic and epigenetic mechanisms shape the evolution of gene regulation

13:35 **Ehsan Sanaei**

Wolbachia in scale insects: A unique pattern of infection prevalence, high genetic diversity, and host shifts

13:51 **Alejandro Obispo Valencia**

Associating gene expression dynamics to the development of the gregarious phenotype in the main pest locust

14:07 **Somia Saadi**

Comparative study on the gene expression levels in the gonads of the desert locust Schistocerca gregaria in gregarious (outbreak) and solitarious (normal) phases

14:23 **Roland Zimm**

Evolutionary lessons from a theoretical developmental morphospace of shark teeth

14:31 **Emma Gairin**

Modelling the development of pigmentation patterns during zebrafish and clownfish metamorphosis

14:39 **Coffee**

15:00 **Luisa Pallares**

Genotype-by-Environment interactions are key for the understanding of the dynamic nature of genotype-phenotype maps

15:35 **Ekaterina Osipova**

Loss of a key muscle gluconeogenic enzyme contributed to the evolution of adaptive metabolic traits in hummingbirds

15:51 **Tânia Paulo**

Mechanisms underlying adaptation of D. melanogaster against oral infection with P. entomophila – genomic versus transcriptomic approaches

16:07 **Amanda Glaser-Schmitt**

Dynamics and stage-specificity of coding and non-coding gene expression during Drosophila melanogaster larval development

16:23 **Saudat Alishayeva**

The role of the adaptive evolution on the non-coding regulatory sequences during ascidian embryogenesis

16:31 **Arnaud Martin**

Gephebase: knowledge integration of genotype-to-phenotype variation in eukaryotes

16:39 **Coffee**

17:00 **Discussion and Social Evening**

Thursday, March 3, 2022

13:00 **Hanh Vu**

Size matters: How does the planarian flatworm know it has grown enough?

13:35 **Miriam Merenciano**

The interplay between developmental stage and environment determines the adaptive effect of a natural transposable element insertion

13:51 **Ting-Hsuan Lu**

Identification of a novel subnetwork involved in eye size variation between Drosophila melanogaster and D. mauritiana

14:07 **Linh Dang**

Integration of functional genomics data to molecularly characterize eye size variation between D. americana and D. novamexicana.

14:15 **Gordon Wiegleb**

Gene expression divergence during Drosophila head development on single nuclei resolution

14:23 **Konstantina Filippopoulou**

Evolution of development of neuronal diversity

14:31 **Sonja Prohaska**

Modelling histone modification dynamics supports histone epigenetics as a driving force of cell differentiation

14:39 **Coffee**

15:00 **Nikola-Michael Prpic Schäper**

How do we translate genotype information into phenotype information?

15:35 **Maridel Fredericksen**

QTL study reveals candidate genes underlying host resistance in a Red Queen model system.

15:51 **Henrike Indrischek**

Vision-related convergent gene losses reveal SERPINE3's unknown role in the eye

16:07 **Ellen McMullen**

Disparity in phenotypes: regulation of carbohydrate transport at the blood-brain barrier

16:15 **Memet Gözüböyük**

The Lim homeobox 1 (Lim1) gene and leg development in Drosophila melanogaster

16:23 **Coffee**

17:00 **Discussion and Social Evening**

Friday, March 4, 2022

13:00	**Pavel Tomancak**	
	Evolution of Morphogenesis	
13:35	**Deepak Dharmadhikari**	
	Natural variation of wing pigmentation spot in a population of Drosophila biarmipes	
13:51	**Victoria Sharp**	
	Strobilation and ephyra survivorship in Cassiopea xamachana associating with diverse Symbiodiniaceae species	
14:07	**Maurijn van der Zee**	
	Ecdysone regulates dorsal closure and is the main target of selection for fast embryonic development in Tribolium castaneum	
14:23	**Franziska Krämer**	
	Creation and preliminary phenotype characterization of a stable Tribolium Zerknüllt 1 knock-out line	
14:39	**Coffee**	
15:00	**Final Discussion and Wrap-up**	

Abstracts

(in presentation order)

Wednesday, March 2, 2022

Sonja Grath

Evolutionary Biology, Ludwig-Maximilians-Universität (LMU) Munich

Understanding how genetic and epigenetic mechanisms shape the evolution of gene regulation

We are generally interested in understanding the mechanisms of how one specific genome can give rise to the vast phenotypic diversity we see between individuals or sexes of one species, but also between tissues or even individual cells within one individual. The focus of this presentation is the evolution of gene regulation. Gene regulation is a complex interplay of different genetic and epigenetic mechanisms that involve, for example, gene expression, DNA methylation and chromatin accessibility. Phenotypic diversity is often caused by differences in gene regulation. Next generation sequencing technologies to investigate genome-wide expression, chromatin accessibility or methylation levels, allowed us to identify genes and gene networks that are differentially regulated between different phenotypes. Such studies revealed that there can be a tremendous amount of genetic and epigenetic expression divergence between individuals of one species and even between cells within one individual. Further, the individual contribution of different mechanisms on gene regulation can vary between species. DNA methylation, for example, alters gene expression in all kingdoms of life, albeit methylation levels vary widely, in particular in arthropods. In order to better understand the mechanisms behind gene regulation and their interactions, we use both computational and experimental approaches. The long-term goal is to identify specific molecular pathways that foster gene regulation and gene expression variation and to determine the genetic and epigenetic mechanisms responsible for controlling these pathways. A further goal is to determine the networks of pathways and how interaction and co-expression of several genes together contribute to an organismal phenotype.

Ehsan Sanaei

School of Environmental Science, Griffith University, Brisbane

Wolbachia in scale insects: A unique pattern of infection prevalence, high genetic diversity, and host shifts

Wolbachia is one of the most successful endosymbiotic bacteria of arthropods. It is a master manipulator, modifying its hosts' biology in many ways to increase its vertical (maternal) transmission. Wolbachia can also undergo host shifts that can be mediated by ecological vectors such as shared host plants or parasitoids. By conducting Illumina pooled amplicon sequencing of 59 infected scale insect samples and 16 direct associates of scale insects (including wasps and ants), I determined 63 Wolbachia strains in these species belonging to supergroup A, B and F. Finally, I fitted a Generalised Additive Mixed Model (GAMM) to assess factors influencing Wolbachia sharing among scale insect species. I found strong effects of host phylogeny without any significant contribution of host geography. This finding can explain a large number of reported Wolbachia host-shifting among congeneric species.

Alejandro Obispo Valencia*, Somia Saadi, Noureddine Bakkali & Mohammed Bakkali

Departamento de Genética, University of Granada, Granada

Associating gene expression dynamics to the development of the gregarious phenotype in the main pest locust

The catastrophic effects of locust plagues in many poor countries, especially in south-east Africa, are notorious and well known. Yet, the molecular basis (see gene expression) that results in such striking phenotype is still largely unknown. Phase polyphenism is an extreme case of phenotypic plasticity that involves two opposite and

exclusive phases, in the case of the dessert locust: solitary and gregarious ones. The differences between both phases affect the behaviour, physiology, development, morphology, both size, color as well as the reproduction of the animal. Whereas solitary locusts molt six times in order to reach adulthood and are larger, green, cryptic and less active; after a population outbreak the locusts are smaller, have a yellow and black pattern, active, swarm and develop faster - molt only five times before reaching adulthood. Outbreaks are promoted mostly by environmental, chemical, visual and mechanical stimuli, which after trespassing a certain threshold promote the animal shift from solitarious to gregarious. Recent RNA-seq studies by our group show deep differences in gene transcription levels in the central neuron system of the solitarious and gregarious locusts. However, potential key genes for the triggering and development of the gregarious phenotype remain unknown. We thus considered that two studies need to be done: (1) a comparative time-course experiment for revealing the dynamics of the gene expression changes throughout the development of the gregarious phenotype and (ii) a similar analysis on a non-outbreak grasshopper species in order to filter the results. We thus exposed solitary *S. gregaria* specimens to stimuli known to trigger gregariousness and we sampled at 11 time points. A similar experiment was carried out using *Eyprepocnemis plorans*—a non-pest species. RNAs were sequenced and we are now assembling transcriptomes. Meanwhile, we are developing new techniques for future experiments. We are especially focused on the in-situ hybridization and gene silencing in order to advance our research towards the functional genomics area. Here we present results of what we have done as well as what we are doing and projecting.

Somia Saadi*, Alejandro Obispo Valencia, Noureddine Bakkali & Mohammed Bakkali

Departamento de Genética, University of Granada, Granada

Comparative study on the gene expression levels in the gonads of the desert locust Schistocerca gregaria in gregarious (outbreak) and solitarious (normal) phases

Schistocerca gregaria is the most dangerous of all pest locusts. It poses a major threat to Africa, the Middle East and Asia. The problem is due to the damage that an extremely increased population causes. Such increase is both due to and affects the reproductive biology of the locust. Normally, locusts form part of the ecosystem where they live as solitarious. When the population size increases, the locusts that live in such crowded conditions turn gregarious and almost every aspect of their biology is affected. Among others, locusts see their reproduction affected. Reproduction is key to understanding and dealing with locust outbreaks. Logically, reproduction depends among others of the proper functioning of the gonads. Being the shift from the solitarious to the gregarious phase of the locusts due to changes in gene expression, we envisaged a study on the changes in gene expression level in the gonads of solitarious and gregarious locusts. We are hence carrying out a comparative RNAseq study aimed at highlighting the gene expression differences that distinguish the functioning of the gregarious (outbreak) ovaries from the solitarious ones, as well as those that distinguish the functioning of the testis of the gregarious locusts from those of the solitarious ones. Here we present the experimental design, the milestones reached, as well as the difficulties we face and the expected outcomes of the research.

Roland Zimm

Institute de Genomique Fonctionnelle de Lyon, ENS de Lyon

Evolutionary lessons from a theoretical developmental morphospace of shark teeth

Teeth represent an ideal system to study the diversification of phenotypic traits for four reasons: they are present across most classes of vertebrates, allowing for macro-evolutionary comparisons, their shapes display a conspicuous variation both between and within species, and this variation is typically functional and adaptive. Finally, tooth development unfolds rather independently from surrounding structures and can, therefore, be explored in relative isolation. While most tooth research has been conducted in mammalian species, shark dentitions display a comparably rich, albeit typically more gradual, heterodont variation, whose developmental basis remains largely elusive. In order to elucidate how the dynamic interplay of different developmental factors and mechanisms creates phenotypic differences, we take advantage of a mathematical model of shark tooth

development. Varying its parameters, we reproduce heterodonty in the catshark and generate a theoretical morphospace of tooth diversity. With this morphospace, we then characterize developmental properties that give rise to specific phenotypic transitions, and observe general patterns and features of the relationship between genotypic and phenotypic variation.

Emma Gairin

Okinawa Institute of Science and Technology, Onna

Modelling the development of pigmentation patterns during zebrafish and clownfish metamorphosis

Pigmentation patterns can be studied to shed light on links between genotypes and phenotypes – which genes control pigmentation cell positioning and cell-cell interactions and lead to particular skin patterns? In zebrafish and clownfish, wildtype organisms and mutants with varying pigmentation phenotypes can be studied to shed light on the role of different genes in cell-cell interactions and in the resulting phenotypes. Using mechanisms identified in the literature, the pigmentation patterns of wildtype and mutant zebrafish (five different mutation types) were modelled in discrete- and continuous-space models. These models were then modified to reproduce wildtype and snowflake mutant phenotypes and thus provide the first insights into which mechanisms and cell-cell interactions are involved in the development of clownfish pigmentation patterns, which will be linked in future genome studies to link genotypes and phenotypes.

Luisa Pallares

Friedrich Miescher Laboratory (FML)/MPI for Biology, Tübingen

Genotype-by-Environment interactions are key for the understanding of the dynamic nature of genotype-phenotype maps

Our understanding of the genetic basis of complex traits, including fitness-related traits, is mostly based on mapping studies that identify genotype-to-phenotype associations. Due to the challenges in performing high-resolution mapping experiments, such genotype-phenotype map in usually explored in just one environment. However, evolutionary theory as well as empirical work suggest that such map is often environmentally-dependent, and that such genotype-by-environment (GxE) interactions are key to understanding phenotypic variation. Here, I will explore how large-scale genomics experiments can help us unravel the role that GxE interactions play in shaping phenotypic variation in outbred populations of Drosophila melanogaster. In particular, I will focus on the genetic basis of lifespan and how different dietary conditions shape such genetic architecture, shedding light on the evolutionary history of alleles that reduce lifespan in stressful diets.

Ekaterina Osipova

Senckenberg Institute, Frankfurt am Main

Loss of a key muscle gluconeogenic enzyme contributed to the evolution of adaptive metabolic traits in hummingbirds

Hummingbirds are the only birds that evolved true hovering flight, and they have the striking ability to fuel this energy-demanding activity almost entirely with recently-ingested sugars. The genomic underpinnings of these extreme metabolic muscle adaptations are largely unknown. We generated a long-read based chromosome-level assembly of the long-tailed hermit, a member of a sister clade to most other hummingbirds, and performed a genome-wide screen for genes that have been specifically inactivated in the ancestral hummingbird lineage. This screen identified the loss of FBP2, a key gluconeogenic enzyme that is active in muscles. Loss of FBP2 occurred around a time where energy-demanding hovering flight is thought to have evolved in hummingbirds. Using CRISPR-Cas9 to generate a partial FBP2 knockout in an avian muscle cell line, we show that downregulating FBP2 upregulates glycolytic flux and mitochondrial respiration, coincident with an increased mitochondria number. Furthermore, genes involved in mitochondrial respiration and organization have upregulated expression in flight muscle of hummingbirds. Together, these results suggest that FBP2 loss

was likely a key step in the evolution of metabolic muscle adaptations required for hovering flight, and illustrate how loss of ancestral genes can contribute to phenotypic adaptations.

Tânia Paulo

Instituto Gulbenkian de Ciência, Oeiras

Mechanisms underlying adaptation of D. melanogaster against oral infection with P. entomophila – genomic versus transcriptomic approaches

In nature, hosts suffer strenuous selective pressures by pathogens, which they try to counteract by deploying immune defence strategies. For Drosophila melanogaster these defences encompass behavioural, developmental and physiological dimensions and although much is known about immunity in this model, gaps remain on how evolution can act on these responses.To address this, we have previously selected an outbred population of D. melanogaster against oral infection with its natural pathogen Pseudomonas entomophila. After just 10 generations of selection, almost 100% of the population had higher survival upon infection and this increased survival was maintained, even under relaxed selection, for over 80 generations. Initially we characterized bacterial load dynamics of *P. entomophila* at different timepoints in our populations to find that Evolved flies were clearing the infection faster and more efficiently than its Control counterparts, which pointed to an acquired higher resistance capability. In parallel, and to understand the genetic basis of such rapid and efficient adaptation we performed Pool-Sequencing of the two regimes at different timepoints of the selection experiment. Simultaneously, and considering the maintenance of the adapted phenotype under relaxed selection, we performed RNA-Seq at different timepoints throughout infection, both of whole-body samples and of gut-specific samples. Our results showed distinct differential expression profiles between local and whole-body responses between our two adapted populations, even though there were clear overlaps at the individual candidate gene level, displaying that our selective pressure acted in a holistic manner. Additionally, and in an attempt to further relate genotype with phenotype, we compared the main candidates of the Pool-Seq with the ones obtained through transcriptomic analysis to realize that there were very few intersections, evidencing a level of disparity between genomic changes and subsequent gene expression. Currently we are validating the main candidates of our multiple approaches using RNAi knockdowns and hope to shed light on the contribution of each study to explain our adapted phenotype as well as the relative importance of each identified physiological mechanism to the overall increased survival upon infection.

Amanda Glaser-Schmitt

Evolutionary Biology, Ludwig-Maximilians-Universität (LMU) Munich

Dynamics and stage-specificity of coding and non-coding gene expression during Drosophila melanogaster larval development

Gene expression variation is pervasive across all levels of organismal organization, including development. In Drosophila melanogaster, transcriptional turnover during development is particularly extensive and often rapid. Few studies, however, have examined variation in developmental transcriptional dynamics among populations, or how it can contribute to phenotypic divergence. We examined coding and non-coding gene expression in the fat body of an ancestral African and a derived European population across three developmental stages spanning ten hours of development at the cusp of metamorphosis. Expression divergence between populations was extensive and largely stage-specific, with the late wandering stage being the most divergent. Coding gene expression divergence among genotypes within another derived population at this stage was also extensive, suggesting increased expression variation among genotypes may be a feature of this stage. During this stage, we also detected an excess of lncRNAs upregulated in the derived population, but a dearth of lncRNAs upregulated in the ancestral population during two wandering larval stages, suggesting the importance of higher and more extensive lncRNA expression in the derived population during these stages. Gene expression dynamics across stages were also highly divergent and the temporal breadth of both coding and non-coding gene expression became more restricted in the derived population. Using differential

expression and network analyses, we identified genes with expression associated with population, approximately 10–25% of which showed population differentiation at the sequence level consistent with local adaptation. Taken together, our results shed light on the temporal dynamics of expression variation within and among populations, as well as how this variation contributes to differentiation between derived and ancestral populations.

Saudat Alishayeva

Harvard University, Boston/MA

The role of the adaptive evolution on the non-coding regulatory sequences during ascidian embryogenesis

The recent speciation between *Ciona intestinalis* and *Ciona Robusta* provides an excellent system for studying selection pressures. Despite the highly similar phenotypic characteristics, these two species differ in terms of adaptation to temperature and their geographic distribution. While the distribution of *C. intestinalis* is mostly reserved to the Mediterranean and North-East Atlantic regions, *C. robusta* has adapted to a warmer climate and invaded many geographic zones, including Australia, South America, and Africa. In ascidians, such adaptation to temperature is linked to developmental processes. Despite the abundance of descriptive studies, there is still a gap in understanding the mechanistic and evolutionary origins of adaptations that happen during development. In this project, I assessed the role of positive selection on regulatory elements that are specific to different stages of ascidian development. I used chromatin accessibility data as a metric for defining developmental regulatory elements because open chromatin upstream of the genes is known to be associated with gene regulatory functions, such as gene expression and gene silencing. Apart from identifying which regulatory elements are under positive selection, I also analysed genes that were in close proximity to these regulatory elements. This is the first study to assess the role of adaptive evolution on regulatory elements in the context of ascidian ontogeny.

Arnaud Martin

George Washington University, Washington / USA

Gephebase: knowledge integration of genotype-to-phenotype variation in eukaryotes We developed Gephebase (gephebase.org) over the past decade as way to keep track of the Loci of Evolution and Domestication: in short, the genes and mutations that have been mapped or confirm to underlie a specific trait variation of adaptive or domestic potential. The need for an evolutionarily broad compilation of this literature is urgent and helps developmental biologists to make sense of the genetic loci that underlie morphological evolution (or other traits). In this short talk, I will briefly present the database and its associated challenges.

Thursday, March 3, 2022

Hanh Vu

EMBL Heidelberg

Size matters: How does the planarian flatworm know it has grown enough?

Body size is a defining feature of each and every animal species with important implications for organismal physiology. However, little is known about how animals "sense" how big they are, so that they can tune their physiology accordingly and ultimately stop growing when they have reached the right size. In my talk, I will present our efforts to understand body size sensing in a remarkable animal with extreme body size plasticity: the planarian flatworm *Schmidtea mediterranea*. Instead of having a fixed body size like most adult animals, planarians continuously adjust their size over two orders of magnitude (from 0.5-40mm in length) according to food availability. Based on our observations that body size fluctuations are accompanied by changes in gene expression and multiple facets of organismal physiology (e.g. growth, energy storage and sexual maturation), we hypothesized that planarians have a mechanism to actively sense their body size. By RNA interference screening, we identified the Activin signaling pathway as a key component of the body size-sensing mechanism. Perhaps most strikingly, we found that Activin signaling activity is strongly correlated with body size and changes in said signaling dose-dependently modulate size-dependent gene expression and consequently, physiology. Our data collectively suggest the existence of "magnigens" – substances that concentration-dependently convert system size into gene expression and physiological change – in planarians and perhaps also other animals.

Miriam Merenciano

Institute of Evolutionary Biology (CSIC-UPF), Barcelona

The interplay between developmental stage and environment determines the adaptive effect of a natural transposable element insertion

Transposable elements (TEs) are repetitive DNA sequences with the ability to move along the genome. TEs have been considered a genome-wide source of regulatory elements capable of regulating nearby gene expression. In *Drosophila melanogaster*, the FBti0019985 natural TE insertion has been previously reported to add a transcription start site to the Lime transcription factor. In this work, we performed in vivo reporter assays and gene expression analysis with CRISPR/Cas9 mutants and natural populations to explore the effects of FBti0019985 on Lime expression under different stress conditions and different developmental stages. We found that this insertion acts as an enhancer in the adult stage under immune-stress conditions. Indeed, the deletion of predicted immune-related binding sites in the TE significantly reduces its enhancer activity in infected conditions, confirming that it harbors functional cis-regulatory elements. We also found that the TE upregulates Lime in embryos, however, in this case we could not pinpoint the molecular mechanism. Finally, we found that TE-induced Lime upregulation was associated with tolerance to bacterial infection and with increased egg-to-adult viability probably due to increased glucose release. Our results suggest that different developmental stages and environmental conditions should be tested in order to fully characterize the molecular and functional effects of a genetic variant.

Ting-Hsuan Lu

Developmental Biology, Georg-August-University Göttingen

Identification of a novel subnetwork involved in eye size variation between Drosophila melanogaster and D. mauritiana

Many traits evolve by a combination of variation in many genomic loci with minor phenotypic effects. Moreover, most genes do not act individually, but they are interconnected in gene regulatory networks (GRNs). Revealing variable nodes and modules within GRN, thus has the potential to gain mechanistic insights into phenotypic evolution. The insect head that harbours the compound eyes is a complex quantitative trait that is

highly variable in Drosophila. The formation of the insect compound eye is determined by a complex GRN composed of more than 5,000 genes. To reveal the molecular and developmental basis of natural variation in eye size and head shape, I studied head development in *Drosophila melanogaster* and *D. mauritiana*. Eye size varies in these two species due to differences in ommatidia number and a trade-off between eye size and interstitial head cuticle has been observed. To reveal novel candidate genes, I integrated several unbiased genome wide datasets, such as developmental gene expression (RNAseq), chromatin accessibility (ATACseq) and quantitative trait loci mapping data. This integrative approach unravelled 65 candidate genes, which I validated functionally for their functional involvement in eye development applying an RNA interference screen. Phenotypically relevant candidate genes were used to reconstruct a novel GRN module that contains predominantly genes with variable expression between species. The addition of few extra genes to this network allowed me to propose developmental processes that may be variable. I tested one of these hypotheses functionally to show that Jim, Pnr and Upd are co-expressed during head and eye development, suggesting a novel role of Jim during this process. Overall, my finding shows that a GRN-centric approach is highly powerful to reveal the mechanisms underlying the evolution of complex organ development.

Linh Dang

Developmental Biology, Georg-August-University Göttingen

Integration of functional genomics data to molecularly characterize eye size variation between D. americana and D. novamexicana

The genetic and developmental basis of complex trait evolution remains largely elusive. Differences in the action of gene products regulating developmental processes result in natural variation in adult morphology. Here, we study natural variation in compound eye size between the two fruit fly species *Drosophila americana* and *D. novamexicana*. We link genetic variants associated with differences in head shape and eye size to variation in genome wide developmental gene expression (RNAseq) and gene regulation (ATACseq). This procedure allows revealing high confidence candidate genes for future functional validation experiments.

Gordon Wiegleb

Developmental Biology, Georg-August-University Göttingen

Gene expression divergence during Drosophila head development on single nuclei resolution

Morphological diversification facilitates adaptation to changing environments. The genetic basis of natural variation of morphology however is still elusive. For many animals, eyes are one of the most important sensory organs and vary greatly in size and shape. We are applying single-nucleus RNA-seq (snRNA-seq) to gain in-depth insights into gene expression and regulation dynamics throughout eye development in Drosophila species on single-cell resolution. In holometabolous insects such as Drosophila, many adult organs develop already in the larvae as a so-called imaginal disc. The eye in particular develops from the eye-antennal imaginal disc (EAD). During development, the cells of the EAD differentiate and give rise to the adult compound eye and other head structures such as the ocelli, maxillae, the antennae, and the head capsule. This development is broadly governed by a gene-regulatory network, the so-called retinal determination network. While the central genes of this network are known, the more delicate mechanisms underlying the variation of eye morphology are not yet completely understood. From preliminary work, sister species of *D. melanogaster, D. mauritiana* and *D. simulans*, are shown to differ in eye size and shape, as well as face. We are using these species to gain insight into the genetic basis of these differences and their evolutionary history. To understand these differences in eye size and shape, it is necessary to understand the eye development during which these differences first arise. For some stages of eye-antennal disc development, bulk-RNA-seq data is already available to approach gene expression changes during development. However, since apart from the eye, these discs also give rise to the antennae, maxillary palp, and parts of the head capsule, it is crucial to be able to differentiate between the individual compartments of the disc. Single-nuclei RNA sequencing can yield both the temporal and spatial information required to gain deeper insights into eye development as it allows assigning gene expression reads gathered in each time point to individual cells. To cover eye development from the onset of eye differentiation

to pupariation, we sampled from five developmental timepoints. These timepoints are chosen to cover major developmental events from growth and proliferation to differentiation. To understand the genetic bases of differences in eye size, shape, and evolution, we additionally performed single-nuclei RNA sequencing the two sister species of *D. melanogaster, D. mauritiana* and *D. simulans*. Since these two species differ in eye morphology but are otherwise closely related. We collected data for the same five time points during larval development. This way, we aim to contribute to understanding the genetic mechanisms that underlie the variation in eye morphology.

Konstantina Filippopoulou

Institut Jacques Monod, Paris

Evolution of development of neuronal diversity

Understanding how neuronal diversity arises can inform our perception of brain function and improve our understanding of neurodevelopmental defects. Temporal patterning of neuronal stem cells is a universal mechanism for the generation of neuronal diversity. It consists of the sequential expression of transcription factors, each expressed for a specific time window, which underlies the proliferation of neural stem cells and leads to the specification of different neuronal types. Temporal transcription factors are involved in complex regulatory networks that allow the temporal series to progress. Single-cell mRNA sequencing has provided unprecedented insight into the transcriptome of individual neural stem cells and facilitated the uncovering of temporal transcription factors. We will apply a comparative single-cell mRNA sequencing approach to the developing optic lobe of different insects, combined with classical genetics and CRISPR, to investigate a) how temporal patterning evolved, b) how new neuronal types emerge through modifications of their temporal series and c) how neurons integrate spatial, temporal, and other inputs to acquire their terminal identity. This unbiased approach can overcome species-specific limitations, reveal conserved principles in neuronal development and give insight in the evolution of neuronal cell types.

Sonja Prohaska

Leipzig University

Modelling histone modification dynamics supports histone epigenetics as a driving force of cell differentiation

DNA replication imposes substantial changes to chromatin. Segregation of parental histones during mitotic replication and replenishment with newly synthesized histones give rise to diluted histone modification patterns. Our common understanding is that this poses a challenge or threat to stable propagation of a cell type-specific epigenetic state and, therefore, maintenance of cell identity. However, what seems like a weakness may open a window of opportunity for transitions between epigenetic states in a controllable manner. Current attempts to model histone modification dynamics focus on bistable switching to explain both, stability and clean and quick transitions between two stable states. However, this comes to the cost of randomness concerning the time point of switching and the energetic cost of repeated modification and demodification events. This way, the fluctuation of the system hinders the cell to reach a well defied permanent state and challenges the role of histone modification as a driver of cell differentiation. We have developed a rule-based stochastic simulation system, to investigate histone modification dynamics [Arnold et al., 2013]. It is a general framework based on accurate modelling of the chemical reaction systems using Gillespie's algorithm. Given a set of histone modifying enzymes and their relevant kinetic parameters, this allows us to generate simulation data in order to study the changing modification landscape on a short chain of nucleosomes. Importantly, this is neither restricted to particular applications, such as polycomb/trithorax, nor certain mechanisms, such as bistable switching or spreading of marks by propagation. In our new model, the system undergoes a single, irreversible state transition, corresponding to a single bifurcation in the epigenetic landscape. The actual state transition is triggered by the dilution of parental nucleosomes during replication and enhanced by the enzyme set pushing towards a stable state. In this context, the inherently stochastic

process of asymmetric segregation of parental histones can lead to differentiation in a pseudo-deterministic way. In the daughter cell, which inherits more of the parental state, the enzyme set will drive the system towards reestablishment of the paternal state pattern resulting in cell fate maintenance. In the other doughter cell, inheriting less of the parental state, the same set of enzymes will induce state switching resulting in call fate redetermination. In summary, our new model shows that asymmetric, but still stochastic, seggregation of parental histones can drive cell differentiation independent of external inputs.

Christian Arnold, Peter F Stadler, and Sonja J Prohaska; Chromatin computation: epigenetic inheritance as a pattern reconstruction problem. J Theor Biol. 2013 336:61-74

Nikola-Michael Prpic Schäper

Allgemeine Zoologie und Entwicklungsbiologie, Justus-Liebig-Universität Gießen

How do we translate genotype information into phenotype information?

The phenotype of an organism is thought to be encoded in its genotype and to be implemented by developmental mechanisms during embryonic and postembryonic development of an individual organism. Developmental biological, genetical and biochemical studies aim at identifying the function of developmental genes and elucidate their role in building the phenotype. There seems to be the implicit notion that by sequencing the entire genome and uncovering all developmental mechanisms the phenotype can be predicted from the genotype. I ask if this is a valid idea and how far current research might be off the mark.

Maridel Fredericksen

University of Basel

QTL study reveals candidate genes underlying host resistance in a Red Queen model system

Parasites and their hosts often interact in very specific ways, in which infectivity and resistance traits depend on the combination of host and parasite genotypes. These specific interactions can lead to balancing selection, maintaining genetic diversity over evolutionary time. However, the molecular underpinnings of the genotypic interactions are largely unknown. Here, we investigate the genetic basis of resistance in the crustacean *Daphnia magna* to its bacterial parasite, *Pasteuria ramosa*. We use QTL analysis and fine mapping to localize the resistance polymorphism to a 28.8-kb region, directly adjacent to a previously identified resistance gene. We compare this 28.8-kb region in the two QTL parents to identify differences between resistant and susceptible genotypes. We identify 13 positional candidate genes, which we narrow down to eight biological candidates on the basis of differential gene expression. These candidates include a fucosyltransferase gene, putative sugar-binding genes, and Cladoceran-specific genes belonging to a large family that is represented throughout the host genome. Our results are consistent with previous studies on host resistance in this system as well as with known mechanisms in other systems, suggesting that parasite spore attachment is mediated by changes in glycan structures on the host cuticle. The Cladoceran-specific candidates suggest that this host lineage may have evolved a resistance strategy that relies on gene duplication. Our results add a new locus to a growing genetic model of resistance in the *D. magna – P. ramosa* system, and they expand our understanding of host–parasite specificity more generally.

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Vision-related convergent gene losses reveal SERPINE3's unknown role in the eye

Despite decades of research, knowledge about the genes that are important for development and function of the mammalian eye and that are involved in human eye disorders remains incomplete. During mammalian evolution, mammals that naturally exhibit poor vision or regressive eye phenotypes have independently lost many eye-related genes. This provides an opportunity to predict novel eye-related genes based on specific

evolutionary gene loss signatures. Building on these observations, we performed a genome-wide screen across 49 mammals for functionally uncharacterized genes that are preferentially lost in species exhibiting lower visual acuity values. The screen uncovered several genes, including SERPINE3, a putative serine proteinase inhibitor. A detailed investigation of 381 additional mammals revealed that SERPINE3 is independently lost in 18 lineages that typically do not primarily rely on visioneyesight, predicting a vision-related function for this gene. To test this, we show that SERPINE3 has the highest expression in eyes of mouse and zebrafish. In the zebrafish retina, serpine3 is expressed in Mueller glia cells, a cell type essential for survival and maintenance of the retina. A CRISPR-mediated knockout of serpine3 in zebrafish resulted in alterations in eye shape and defects in retina layering. Furthermore, two human polymorphisms that are in linkage with SERPINE3 are associated with eye traits. Together, these results suggest that SERPINE3 has a role in vertebrate eyes. More generally, by integrating comparative genomics with experiments in model organisms, we show that screens for specific phenotype-associated gene signatures can predict functions of uncharacterized genes.

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Disparity in phenotypes: regulation of carbohydrate transport at the blood-brain barrier

The brain is a highly energy-demanding organ, requiring a constant energy supply, most of which is acquired from dietary metabolites. At the same, the brain must be protected from the circulation to prevent the influx of harmful substances, which may disturb neuronal homeostasis. This separation of the brain from the blood, or hemolymph in the case of Drosophila melanogaster, is mediated by the blood-brain barrier (BBB). The tight regulation of the BBB necessitates the expression of transporters to facilitate the uptake of metabolites, such as carbohydrates, into the brain where they can be utilized as neuronal fuel. We characterized three BBB transporters, Tret1-1 and MFS3 (Major Facilitator Superfamily Transporter 3), located in perineurial glial cells, and Pippin, found in both the perineurial and subperineurial glial cells. All three transporters facilitate uptake of circulating trehalose and glucose into the BBB-forming glial cells. RNA interference-mediated knockdown of Pippin and MFS3 leads to pupal lethality and knockdown of Tret1-1 results in locomotive defects. Conversely, pippin and Mfs3 null mutants reach adulthood, although they do show reduced lifespan and activity, whereas Tret1-1 mutants are pupal lethal. We show that carbohydrate transport efficiency, and resulting lethality, found upon loss of MFS3 or Pippin is rescued by the compensatory upregulation of Tret1-1 in pippin and Mfs3 null mutants, while RNAi-mediated knockdown is not compensated for. This demonstrates that the compensatory mechanisms in place upon mRNA degradation following RNA interference can be vastly different from those resulting from a null mutation.

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The Lim homeobox 1 (Lim1) gene and leg development in Drosophila melanogaster

In my study, I aimed to detect other genes with which the Lim1 gene interacts during leg development. In order to reveal this interaction, I conducted a genome-wide association study (GWAS). I performed the GWAS using the whole genome sequenced DGRP strains and the online software available at the DGRP2 website. In the study, 122 DGRP strains were crossed with both the strain in which the Lim1 gene was mutant and the control strain (BL 6326 w1118) in which this mutant was produced. The length and width measurements of both the femur and tibia of the three legs of the individuals obtained from these crosses (only females since Lim1 is inherited on the X chromosome) were performed. When these measurements were evaluated statistically, the interaction terms ""Genotype"" (Lim1, Control), ""Strain"" (DGRP) and ""Genotype x Strain"" showed high significance as a result of analysis of variance. As a result of GWAS, many candidate genes were identified that were not previously associated with leg development. Currently, validation studies of these candidate genes are ongoing.

Friday, March 4, 2022

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Evolution of Morphogenesis

tba

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Natural variation of wing pigmentation spot in a population of Drosophila biarmipes

Continuous variation in morphological traits is the raw material for natural selection. Such morphological variation within a population may drive or accompany speciation. It is therefore of interest, to not only look at genetic makeup of an organism but also to be able to precisely measure and quantify the phenotype and correlate it with its respective genotype. While we have base-pair level resolution of genetic makeup for hundreds of species, the same cannot be said about phenotypic quantification. Particularly, for traits that vary in several dimensions. In this study, we present a pixel by pixel analysis of a population of *Drosophila biarmipes*, a Drosophilid in which males present a melanised spot on its wing blade. To assess the intra-specific variation of this trait, we look at a large population of wild isofemale lines captured in India. We align tens of wings from a hundred isofemale lines to a reference wing based on cross-vein landmarks. We then look at pixel by pixel values of each wing allowing us to quantify variation in pigmentation intensity, size etc., while retaining the spatial information. With this approach, we identify several clusters of lines that are distinct from each other and which occupy different corners of morphospace. We show that these phenotypes are stable under laboratory conditions nearly six years apart. These morphologically-distinct set of lines allow access to their respective phenotypes and thus directly to their genetic make-up potentially increasing the resolution of Genotype-Phenotype correlation studies to follow.

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Strobilation and ephyra survivorship in Cassiopea xamachana associating with diverse Symbiodiniaceae species

Cassiopea xamachana, the upside-down jellyfish, is a model system for studying host-symbiont relationships in marine organisms. Like ecologically important coral species, Cassiopea has a mutualistic relationship with algae in the family Symbiodiniaceae, but the host is unique in its symbiosis-driven metamorphosis. *C. xamachana* juvenile polyps only reach adulthood (through a process called strobilation) upon successful establishment of symbiosis with algae. In the wild, *C. xamachana* predominantly associates with one homologous symbiont but can be artificially infected with foreign algae species in a lab setting. Several Cassiopea studies have focused on this unique symbiont flexibility and used strobilation as the marker for successful mutualism. However, no current study had looked at the survivorship of adults post-strobilation, or potential changes in host phenotype that might arise from these forced associations. My study follows infection of *C. xamachana* polyps with 22 algae symbionts, whether strobilation occurs, and the survivorship of the host. As marine communities change as a consequence of anthropogenic activity and climate change, it is of utmost important for conservation of diverse marine symbiotic organisms to understand how changes in symbiont associations relate to survivorship of the host.

Maurijn van der Zee

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Ecdysone regulates dorsal closure and is the main target of selection for fast embryonic development in Tribolium castaneum

Climate change exerts strong selection on insect developmental speed, due to mismatches with host plant or prey availability. Here, we investigated the genetic basis of embryonic developmental speed by selecting replicate outbred populations of the beetle *Tribolium castaneum* for fast or slow development during 20 generations. The response of selection was spectacular, and the selection lines started to diverge in developmental speed during dorsal closure. Pooled Illumina and nanopore resequencing, combined with RNAseq, qPCR and a small RNAi screen, revealed two main targets of selection. First, a region upstream of melted, a protein involved in insulin signaling. And second, a 230 bp deletion upstream of CYP18A1, a cytochrome that degrades active ecdysone. This deletion contains a binding site for the architectural protein Tramtrack affecting chromatin conformation. Using CRISPR-Cas9 technology we recreated this allele in the homogeneous genetic background of the Georgia lab strain, and demonstrate that this precipitates the ecdysone peak inducing dorsal closure. This allele in the fast lines explains 50% of the difference with the unselected lines. Thus, the response to selection on developmental speed seems to be mediated by 2 alleles of large effect, rather than many alleles of small effect. With this study, we have revealed natural genetic variation in ecdysone signaling that may be highly relevant in response to global warming, for instance to match caterpillar hatching with host plant bud burst.

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Creation and preliminary phenotype characterization of a stable Tribolium Zerknüllt 1 knock-out line

Extra-embryonic membranes are believed to be a significant factor for the evolutionary success of insects. However, the fruit fly *Drosophila melanogaster*, unlike the majority of insects, develops only a single, dorsally located, vestigial and hence functionally limited membrane, the amnioserosa. In consequence, the red flour beetle *Tribolium castaneum* has become a frequently used model to investigate the morphogenetic principles and function of extra-embryonic structures. In this species, formation of the amnion and serosa, two distinct extra-embryonic tissues, has already been associated with several genes, for example Zerknüllt 1. However, the functional spectrum of this gene has not yet been fully characterized. Currently, data are only available from parental RNAi-based knock-down experiments (van der Zee et al. 2005, Current Biology; Panfilio et al. 2013, Biology Open; Jain et al. 2020, Nature Communications). However, Zerknüllt 1 has never been investigated at the gene level, and wild-type as well as knock-down development have never been compared with a knock-out phenotype. We have combined CRISPR/Cas9 genome editing (Gilles et al. 2015, Development) with our AGOC vector concept (Strobl et al. 2018, eLife) to create a stable Zerknüllt1 knock-out *Tribolium* line via insertional mutagenesis. In detail, we inserted 3×P3-based eye marker cassettes via homology-directed repair into the Zerknüllt 1 coding sequence to impede proper expression. Preliminary imaging data of DAPI-stained homozygous knock-out embryos suggest a similar phenotype as found in previous knock-down experiments, i.e. the embryos appear to be lacking a serosa and are covered only by a dorsal amnion. Next, we will generate double transgenic lines that also carry fluorescent protein expression cassette suitable for live imaging experiments to dynamically characterize the knock-out development cascade during gastrulation.

Developmental Biology section of the German Zoological Society (DZG)

Mind the gap: From Genotype to Phenotype and the role of Modelling, Genomic Prediction, and Development

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