

March 17th- 20th 2025

## **ABSTRACT BOOK**

### **General information**

#### **Congress Venue**

The 3<sup>rd</sup> Münster Evolution Meeting 2025 will take place in the Schloss – the main university building.

Address: Schlossplatz 2, 48149 Münster

Google maps link: <a href="https://maps.app.goo.gl/Jt6YWo3iEa3BmrZ46">https://maps.app.goo.gl/Jt6YWo3iEa3BmrZ46</a>

#### **Registration and Information**

The registration desk is located in the foyer of the Schloss close to the main entrance. If you have any questions, feel free to ask there.

#### **Washrooms**

Washrooms are located in the basement.

#### WiFi

Visitors of the University of Münster can register for the university's network via eduroam, which enables a visitor from one participating institution to gain network access at another. A prerequisite is that the home institution takes part in the eduroam project.

WiFi (SSID): Eduroam

UserID: username@domain (e.g. if your username is darwin123 and your home

institution is the University of Cambridge, you would enter

darwin123@cam.ac.uk)

Password: Use the password you use to access services at your home institution.

Guests from other institutions which are not a member of the eudroam project can log in to the open WiFi **GuestOnCampus**, which has a daily data limit of 1 GB per device.

#### **Talks**

All talks will be held in **Aula** or **S1**. Oral presentations will either be 15 minutes long including two to three minutes for questions/discussion or 30 min long including five to ten minutes for questions/discussion.

**Submitting files for your talk:** Preferred file format is **pdf** or **PowerPoint**. No other file formats are accepted. Please bring your files on a USB stick and hand them to us ideally one day before your presentation or latest, during the coffee/lunch break before your session. The presentation computers will be running Windows 10 and are equipped with PowerPoint 2016 and Adobe PDF reader. If there are videos embedded in the presentation, make sure

they are uploaded correctly. Please do not consider projecting your presentation from your own laptop, as this will inevitably cause delays.

#### **Posters**

Poster boards are set up in the foyer opposite to the registration desk. The posters should be prepared in portrait format in ISO A0 size (119 cm x 84 cm). There are two poster sessions: Monday, 17.03.2025 (18:00-20:00) & Tuesday 18.03.2025 (18:00-20:00). For poster numbers and further information, see page 6 in this book.

**Poster Flash talks:** Each poster presenter will have the opportunity to promote their poster during the flash talk session on Monday or Tuesday. Please prepare one slide, and you will have one minute to pitch your poster. The slides can be submitted during registration or latest by the end of the last coffee break.

#### **Abstracts**

The abstracts of all talks and posters can be found in the book, order by the last name of the presenting author.

#### Catering

Coffee, tea and snacks are available in the foyer from the first coffee break until the last coffee break. For lunch, there are various opportunities to have lunch in the vicinity of the venue. During the poster session, self-serving finger-food buffets with vegetarian and vegan options and beverages are offered.

#### **Public lecture**

On Wednesday evening (17:00 - 18:30) Prof. Dr. Lena Wilfert from University of Ulm will give a public lecture on the topic **Bestäuber im Anthropozän: die Evolution von Bienen und ihren Mikroben im globalen Wandel**.

Abstract: Der globale Wandel – von Habitatsverlusten zu Umweltverschmutzung und Erderwärmung – bedroht die Gesundheit und den Bestand vieler Arten, insbesondere auch des Menschen. Dies geschieht meistens dadurch, dass sich die Beziehungen zwischen Arten, also zum Beispiel zwischen Pflanzen und ihren Bestäubern oder Wirtsorganismen und ihren Krankheitserregern, zum Negativen verändern. Hier werde ich anhand von Bestäubern, wie den Honigbienen und wilden Hummeln, aufzeigen, wie sich der globale Wandel auf solche Beziehungen auswirken kann und beispielweise zur Evolution von neu-entstehenden Krankheiten führen kann. Solche evolutionsökologischen Störungen von Biotischen Interaktionen tragen dazu bei, dass wir auch bei diesen Nutzinsekten einen starken Rückgang von Arten und Populationen zu beklagen haben. Die aktuelle Forschung zeigt aber auch, wie wir durch konkrete Umweltschutzmaßnahmen Bestäuber unterstützen können, wodurch letztlich die Gesundheit unserer Umwelt und des Menschen unterstützt wird.

Please note that this talk will be in German

# **Schedule Overview**

Monday, 17.03.2025		Tuesday, 18.03.2025		Wednesday, 19.03.2025		Thursday, 20.03.2025	
		09:00-10:55	Talks	09:00-10:40	Talks	09:00-10:25	Talks
		10:55-11:30	Coffee	10:40-11:15	Coffee	10:25-11:00	Coffee
		11:30-12:30	Talks	11:15-12:15	Talks	11:00-12:30	Talks
	Registration		Lunch(self		Lunch(self		
12:00-13:00	& Coffee	12:30-14:00	organised)	12:15-14:00	organised)		
13:00-15:10	Talks	14:00-15:30	Talks	14:00-15:10	Talks		
	Coffee &		Coffee &		Coffee &		
15:10-15:45	Cake	15:30-16:00	cake	15:10-15:45	cake		
15:45-17:45	Talks	16:00-17:45	talks	15:45-16:30	talks		
	Poster		Poster				
18:00-20:00	session	18:00-20:00	session	16:30-17:00	break		
					public		
				17:00-18:30	lecture		
					DZG event		
					for Grad		
				19:00-21:00	students		
					Networking		
					event:		
					Open to all		
				21:00- open	MEM		
				end	participants		

For detailed version & updates, visit our homepage:



# **Poster numbers**

Monday	, 17.03.2025	Tuesday, 18.03.2025			
Poster Number	Name	Poster Number	Name		
1	Aryadevi Anitha Shaji	22	Maximilian Baldy		
2	Margaux Aubel	23	Karl Kaether		
3	Eva Adina Baumgarten	24	Julia Leman		
4	Paula Bongers	25	Sarah Lucas		
5	Inês Borges	26	Stylianos Mavrianos		
6	Nathalie Brenner	27	Christoph Mayer		
7	Nina Casillas	28	Sidra Tul Muntaha		
8	Tania Chavarria	29	Zeynep Önder		
9	Ioannis Chrysostomakis	30	Temitope Oriowo		
10	Jasmin Cichy	31	Olena Orlova		
11	Amelie Deynet	32	Johanna Pieplow		
12	Elias Dohmen	33	Anjana Prasad		
13	Liliya Doronina	34	Leopold Preuß		
14	Eswarrijah Eswaran	35	Tobias Prüser		
15	Lukas Franke	36	Kim Rohlfing		
16	Diana González	37	Alina Schüller		
17	Kevin Hsiung	38	Pratyaksh Singh		
18	Xuejing Hu	39	Raphael Steffen		
19	Sahar Javaheri Tehrani	40	Nils Sternberg		
20	Carsten Kemena- Rinke	41	Eckart Stolle		
21	Jos Kramer	42	Benedikt Treml		
		43	Nanda Vo		

# **Abstracts**Talks & Posters

Ordered by last name of the presenting author

### Genomic Insights into Biodiversity Decline: Integrating Reference Genomes and Population Genomics within the BIGFOOT Project

<u>Aryadevi Anitha Shaji</u><sup>1</sup>, Elisabeth Karalashvili<sup>1</sup>, Nina Casillas<sup>1</sup>, Eckart Stolle<sup>1</sup>, Astrid Böhne<sup>1</sup>, Ann-Marie Waldvogel<sup>2</sup>

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The accelerating global decline in biodiversity, evident in IUCN and Germany's own Red Lists, underscores the urgent need for refined conservation strategies. Although traditional biodiversity conservation methods have focused on habitat preservation, recent research suggests that genomic data can reveal critical patterns of population decline not detectable with conventional tools. This study is part of the BIGFOOT (Biodiversity Decline's Genomic Footprint) project, a multi-institutional initiative aimed at monitoring and protecting species threatened by extinction in Germany by leveraging genomics and ecological modelling. Here, we employ genomic resources to study genomic signatures of population decline in ten species including insects, vertebrates, and mollusks, that are often-overlooked in conservation genomics studies. We are generating annotated, chromosome-resolved reference genomes for five species, including Great capricorn beetle and bush cricket, along with whole-genome datasets from historic and current populations of ten species sampled across Germany and from museum collections. By analysing species from varied ecosystems with unique adaptive traits, distinct habitat dependencies, and differing levels of habitat loss and ecological isolation, we aim to encompass a comprehensive range of genetic and ecological responses to environmental and anthropogenic pressures over time. Importantly, comparative analysis between these past and present populations will reveal patterns of population decline; integrating these insights with genomic forecasting will support the development of more effective, genomics-based conservation strategies.

<sup>&</sup>lt;sup>1</sup> Leibniz Institute for the Analysis of Biodiversity Change, Bonn, Germany

<sup>&</sup>lt;sup>2</sup> University of Cologne, Germany

### Ali-U-Net: A Convolutional Neural Network for Multiple Sequence Alignment of DNA Sequences

Petar Arsic<sup>1</sup>, Christoph Mayer<sup>2</sup>

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Two major trends are shaping the analysis of biological and medical data. Datasets are growing rapidly in size, and machine learning is revolutionizing how they are analyzed. An important problem in molecular biology is that of aligning multiple nucleotide sequences. Here we report a convolutional neural network that is capable of aligning nucleotide sequences with an accuracy close to and in some scenarios even better than state-of-the-art software alignment tools such as MAFFT, MUSCLE, TCOFFEE, and CLUSTAL-Omega. Our alignment neural network attains high accuracies on test data sets that are challenging even for conventional software while being faster or about equally fast on a single CPU core. Even though the algorithm currently works with fixed-size alignment windows of the size 48x48 or 96x96 nucleotides, we are confident that this limitation will be overcome soon.

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<sup>&</sup>lt;sup>2</sup> Museum Koenig Bonn, Germany

#### Selection Patterns Across Vertebrates in a Sperm Ion Channel

Margaux Aubel<sup>1</sup>, Alina Berger<sup>1</sup>, Erich Bornberg-Bauer<sup>1,2</sup>

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Several chemical reactions need to take place inside the sperm cells before the fertilisation of the egg cell or oocyte can occur. Just before fertilisation the sperm cells or spermatozoa become hyperactivated and move faster. Hyperactivation enables the sperm to break through the oocyte's barriers to accomplish fertilisation. This hyperactivaton is caused by the influx of calcium ions into the spermatozoa tails. The influx of calcium ions is accomplished through the sperm-specific ion channels known as CatSper. The CatSper ion channel is selective for calcium and located in the tail of the spermatozoa. Successful fertilisation of the oocyte depends on the correct function of the CatSper ion channels embedded in the membrane. The channel has been shown to be essential for male fertility in humans, mice and other mammals. In several cases, male fertility issues have been shown to be caused by non-functional CatSper proteins. The CatSper complex comprises 14 different subunits, most of which are present not only in mammals but across the vertebrate phylogeny meaning they emerged around 500 million years ago. Here, we identify 1) the presence and absence of the single subunits across vertebrates, 2) gain and loss of domains within the subunits, and 3) patterns of positive selection present in single species and larger phylogenitic units. We find distinct patterns of positive selection for the single subunits in different vertebrates, most notably in primates. In line with previous research, we found a gain of disordered domains in mammals compared to other vertebrates.

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<sup>&</sup>lt;sup>2</sup> Department of Protein Evolution, MPI for Developmental Biology, Tuebingen, Germany

### Cortisol and Testosterone levels in different social environments in male guinea pigs in different stages of the ontogeny

Maximilian Baldy<sup>1</sup>, Helene Richter<sup>1</sup>, Sylvia Kaiser<sup>1</sup>

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My research project is, broadly, about the effects of different social environments on endocrine parameters and behaviour, as well as the consequences of these parameters for reproductive success in male guinea pigs (Cavia aperea f porcellus). My experiments can be summarised thusly: two groups of male guinea pigs were each held in pairs with a female, with one group receiving weekly social stimulation through other guinea pigs, and the other group not receiving this treatment. The animal's cortisol reactivity and testosterone levels were regularly determined through blood samplings from the juvenile phase (post-weaning) until the adult phase. As soon as all data is collected (early next year) analysis will begin. My poster would contain the first results of my project, a thorough analysis of the hormonal data using linear mixed effects models and repeatability analysis. Analyses will include measurements of weight and weight changes in the test situation, cortisol basal and response values as well as relative and absolute changes in cortisol levels, exact age at the timepoint of the test, and testosterone. The results will then be interpreted in the light of relevant concepts such as social niche and niche conformance. Whatever the results may reveal, the results will be important for the examination of the mechanisms of social niche conformance and the importance of the timepoint of social niche conformance specifically in male guinea pigs.

### Evolution of trait variance and its consequence on species evolutionary response in complex mutualistic networks

Gaurav Baruah<sup>1</sup>, Meike Wittmann<sup>1</sup>

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Climate change, especially rising temperatures, poses a threat to biodiversity and ecosystem resilience by disrupting phenological synchrony in plant-pollinator networks. These interactions, essential for ecosystem stability, are highly sensitive to temperature shifts, which can lead to mismatches in plant flowering and pollinator emergence, weakening species interactions and reducing resilience to environmental disturbances. In this study, we model species' evolutionary responses to an abrupt temperature increase, examining onedimensional adaptation in mean phenological trait versus multi-dimensional adaptation involving both the mean and trait variance. Our results indicate that networks where species can evolve in both dimensions exhibit greater resilience, especially those species with a larger number of interactions that enhance genetic variance and exhibit evolutionary rescue under strong selective pressures. Even specialist species benefit from increased resilience through interactions with highly connected generalists, which help stabilize adaptation of entire mutualistic network to a new environmental optima. We observed the emergence of opposing selection pressures in complex networks which consequently promote increased genetic variance, or "evolvability," enabling faster adaptation, and thus reducing trait lag, and boosting species survival. These findings underscore the critical role of evolving genetic variance and network structure in mitigating the impacts of phenological mismatches to rising environmental temperatures. This study provides valuable insights into the adaptive capacity of mutualistic networks, highlighting the urgent need for conservation efforts in the face of accelerating climate change.

#### Population Genomics of Mediterranean Bombus terrestris Subspecies

Eva Adina Baumgarten<sup>1,2</sup>, Nathalie Brenner<sup>2</sup>, Thomas Joseph Colgan<sup>1</sup>, Eckart Stolle<sup>2</sup>

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The evolutionary trajectories of species have been shaped by the rising selective pressures exerted by changing climates over recent decades. Understanding how species respond to these forces is of utmost importance, as shifts in their distribution and abundance have been observed both locally and globally. Bumblebees, highly valued for their ecological and economic roles as pollinators, represent a particularly diverse group with a wide geographic distribution including considerable subspecies radiation. One driver of subspecific speciation can be a geographic separation between islands and the mainland. On islands, populations are under local selective pressures and shaped by low genetic diversity, reduced gene flow, and increased inbreeding. To understand these genetic variations between separated locations, we examined how four different Mediterranean Bombus terrestris subspecies have adapted to their local environments. We used whole-genome sequencing data to investigate their population genomics including genetic differentiation and structure, signatures and extent of selection, and adaptive potential. Our analysis suggests that gene flow is ongoing between subspecies, likely facilitated by the proximity of the studied islands and the extensive dispersal capabilities of bumblebees. Consequently, we identified genomic regions of introgression between subspecies. However, we found that more isolated island subspecies suffer from elevated inbreeding rates, genetic drift, and reduced genetic diversity despite presumably large initial population sizes. Interestingly, the proximity of subspecific populations and the resulting increased gene flow, which would typically reduce genetic differentiation, did not alter distinct phenotypic characteristics, suggesting that selective pressures may be favouring these traits. Using genomic differentiation, and analysis of divergent forms of selection, we identified genomic regions, which may contribute to local adaptation and speciation. Our findings enhance our understanding of how populations, specifically bumblebees are shaped by evolutionary processes and how isolation on islands is facilitating speciation and local adaptation.

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### Innovations, reductions, co-evolution – how land plant sexual reproduction gained independency from water

#### Annette Becker<sup>1</sup>

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The transition of water-dependent reproduction of algae to water-independent mechanisms in many land plant lineages allowed plants to colonize diverse terrestrial environments, leading to the vast variety of plant species. An emergence of modified cell types, novel tissues, and organs enabled this transition; their emergence is associated with the co-evolution of novel or adapted molecular communication systems and gene regulatory networks. In the light of an increasing number of genome sequences in combination with the establishment of novel genetic model organisms from diverse green plant lineages, our knowledge and understanding about the origin and evolution of individual traits that arose in a concerted way increases steadily. I will first provide a comprehensive overview on the origin and evolution of reproductive novelties such as immobile sperms, ovules and seeds, carpels, and how they generated the need for novel communication systems and double fertilization. I will then go deeper into the molecular evolutionary processes that shape the carpels, which, after fertilization will develop into fruits.

#### The Evolutionary Dynamics of Novel Endosymbiosis

#### Lutz Becks<sup>1</sup>

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Stable endosymbiosis, where one organism lives within another, is common across the tree of life, yet understanding its evolution remains a significant challenge. A major difficulty in studying endosymbiosis lies in determining when natural selection favors the integrated unit of the host and its endosymbiont over the free-living forms of the symbiotic partners. Additionally, understanding the processes that drive the shift from independent to symbiotic integration is a complex and ongoing puzzle. To investigate this, we conducted an experimental evolution study using ciliate-algal systems. In our experiment, we paired different ciliate and algal species that varied in their initial ability to form endosymbiotic relationships. Experimental lineages were initiated with pairs where: neither the ciliate nor the algae could form endosymbiosis, only one partner had the ability to form endosymbiosis, or both partners could already form endosymbiotic relationships. This design allowed us to examine how novel endosymbioses could evolve in the first two scenarios. Our results revealed that novel endosymbiosis evolves repeatedly, primarily under stressful environmental conditions with elevated energy demands, but not in more benign environments. The evolution of endosymbiosis was driven by the fitness benefits it provided to both the ciliate host and the endosymbiotic algae, but it required adaptive changes in both partners, even in lineages where one partner was already capable of forming endosymbiosis at the start of the experiment. By comparing lineages over time and across different conditions, we are now studying the evolving mechanisms at the genomic level, as well as in physiology, morphology, and other phenotypic traits. This approach will provide deeper insights into the processes driving the evolution of endosymbiosis.

#### Diversity of sex chromosomes in fish

Sopie Smith<sup>1</sup>, Kevin Hsiung<sup>1</sup>, Arseny Dubin<sup>2</sup>, Olivia Roth<sup>2</sup>, Astrid Böhne<sup>1</sup>

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Sex determination is highly variable in its determinants and can be driven by different environmental and genetic factors. Genetic sex determination includes many different types of sex chromosomes. While the detection of sex chromosomes has seen a tremendous uprise with the advance of sequencing data, our knowledge on driving forces and mechanisms of sex chromosome emergence and turnover is limited. Changes in sex determination systems are particularly frequent in fish, a main model system of our research. Within the hyperdiverse fish family Cichlidae we detected an unprecedented high rate of sex chromosome turnover involving different chromosomes and yet non-random sex chromosome recruitment. We demonstrate cases of sex chromosome turnover with and without changes in heterogamety. We demonstrate that in addition to inter- also intraspecies variability exists in sex chromosome constellations. We explore patterns of sex-biased gene expression and their relation to sex chromosome evolution as well as structural changes on sex chromosomes to ultimately shed light on the mechanisms driving and following sex chromosome turnover.

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### Investigating if the peculiarity of auxotrophy in E. coli is an influential factor in microbial networks interactions

Paula Bongers<sup>1</sup>, Shryli Kedambadi Shreekar<sup>1</sup>, Christian Kost<sup>1</sup>

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Microbial networks are shaped by abiotic factors such as environmental conditions and temperature, as well as biotic factors, including network topology and cross-feeding interactions. To investigate the role of auxotrophy in microbial network stability, we utilize auxotrophic Escherichia coli strains in defined media. We hypothesize that bacterial strains with multiple amino acid auxotrophies will require equivalent amounts of focal amino acids for optimal growth, irrespective of the auxotrophy context. To test this hypothesis, we will use a wild-type (WT) E. coli control and single auxotrophs for 10 amino acids: arginine, histidine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan, and tyrosine. Growth kinetics will be measured across a gradient of each focal amino acid to determine the minimal required concentration for optimal growth. For double auxotrophs, media will be supplemented with the required concentration of the first auxotrophic amino acid determined in the first experiment, and gradients of the second amino acid will be used to evaluate growth dynamics. Our expectations would suggest that differences in amino acid requirements between single and double auxotrophs may be explained by the metabolic pathways involved, particularly interactions with the tricarboxylic acid (TCA) and glycolysis cycles. These findings could potentially highlight the impact of metabolic dependencies on cross-feeding interactions and network stability.

#### Centromere repositioning and size variation as drivers of speciation in Darwin's finches

<u>Inês Borges</u><sup>1</sup>, Niki Vontzou<sup>2</sup>, Erik Enbody<sup>3</sup>, Carl-Johan Rubin<sup>4</sup>, Brian Davis<sup>5</sup>, Leif Andersson<sup>4,5</sup>, Valentina Peona<sup>6,7</sup>, Francisco J. Ruiz-Ruano<sup>1</sup>, Alexander Suh<sup>1,8</sup>

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Centromeres are structural regions of chromosomes critical for accurate chromosome segregation during cell division, acting as the primary binding site of the kinetochore. Despite their critical role, centromeres exhibit considerable variation in size, sequence and position across closely related species and even within the same species, a phenomenon known as the "centromere paradox". The centromere drive hypothesis proposes that centromeres can act like selfish genetic elements and drive non-Mendelian segregation during asymmetric female meiosis, creating bias towards the transmission of larger, stronger, centromeres. This hypothesis helps to explain the rapid evolution of these genomic regions. Simultaneously, differences in centromere position and significant size variation can hinder or disrupt normal chromosome pairing and suppress recombination during meiosis, acting as a barrier to reproduction due to reduced fertility or inviability of offspring between individuals with incompatible differences in centromere position or size, contributing towards speciation. Bird genomes are particularly well-suited to study this phenomenon, as centromere repositionings have been previously identified in some avian species. Additionally, their chromosomes are otherwise highly syntenic and collinear, facilitating the comparative study of the satellite DNArich centromeric regions. In this project, we empirically explore the hypothesis that differences in centromere position and size contribute towards reinforcement of speciation using Darwin's finches as a case study. For this, we identify putative centromere regions in species from this clade with an approach combining satellite DNA annotation, recombination rate estimates and methylation levels. We find extensive differences in centromere size between species and haplotypes in the same individual, as well as some potential centromere repositionings, providing new insights into how their pronounced effects on genetic variation may play a role in the adaptive radiation of Darwin's finches.

#### Virulence Trade-offs in EHEC 0104:H4: The Role of RpoS Polymorphisms

<u>Karla Bosse-Plois</u><sup>1</sup>, Michael Berger<sup>1</sup>, Natalie Scherff<sup>1</sup>, Ulrich Dobrindt<sup>1</sup>, Alexander Mellmann<sup>1,2</sup>, Petya Berger<sup>1,2</sup>

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Polymorphism of the stationary-phase sigma factor RpoS is a well-described adaptive mechanism that enables Escherichia coli (E. coli) and other Proteobacteria to survive in diverse natural conditions. Genes regulated by RpoS are critical for various resilience mechanisms against stressors, including prolonged starvation. Interestingly, mutations that attenuate RpoS might suggest a trade-off between resistance and nutrient scavenging, allowing bacteria to be more resilient or grow more efficiently. Recently, our laboratory identified three single nucleotide polymorphisms (SNPs) in rpoS of the enterohemorrhagic E. coli (EHEC) strain O104:H4 that caused the largest recorded outbreak of food-borne infections in Germany in 2011 with the highest incidence of haemolytic uremic syndrome globally. One isolate harboured a laboratory-acquired mutation in the start codon of rpoS, while two isolates were carrying naturally occurring SNPs in the C-terminal region, resulting in a premature stop codon or a non-synonymous substitution, which is known to influence transcription initiation on relaxed templates. Here, we explore the impact of these SNPs on the virulence of EHEC O104:H4. By recombinant expression of the different rpoS alleles in EHEC O104:H4 Δstx2 ΔrpoS, we demonstrated that RpoS expression was decreased in mutant strains compared to native RpoS, as shown by Western Blots. Additionally, RpoS-regulated catalase activity was severely reduced as well. Next, we observed an increased aggregation phenotype associated with all three rpoS mutations, which correlated with enhanced production of the aggregative adherence fimbriae. Furthermore, we confirmed that production of virulence factors SepA, a serine protease autotransporter, and FliC, a flagellin protein, was upregulated as well. Our findings demonstrate that rpoS mutation results in increased virulence of pathogenic EHEC O104:H4, thus further expanding our understanding the role of RpoS as a master regulator of virulence.

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<sup>&</sup>lt;sup>2</sup> National Consulting Laboratory for Hemolytic Uremic Syndrome (HUS)

### Population genomics and subspecies radiation of the buff-tailed bumblebee across Europe suggest strong impact of islands on speciation

<u>Nathalie Brenner</u><sup>1</sup>, Villu Soon<sup>2</sup>, Toshko Ljubomirov<sup>3</sup>, Pilar de la Rúa<sup>4</sup>, Antonio Felicioli<sup>5</sup>, Paolo Biella<sup>6</sup>, Gaetana Mazzeo<sup>7</sup>, Roberto Catania<sup>7</sup>, Ignazio Floris<sup>8</sup>, Thomas Joseph Colgan<sup>9</sup>, Patrick Lhomme<sup>10</sup>, Christopher Mayack<sup>11</sup>, Martin Husemann<sup>12</sup>, Wilhelm Osterman<sup>13</sup>, Eckart Stolle<sup>1</sup>

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- <sup>5</sup> Department of Veterinary Sciences, University of Pisa, Italy
- <sup>6</sup> Department of Biology and Biotechnology, University of Milano-Bicocca, Italy
- <sup>7</sup> Department of Agriculture, Food and Environment, University of Catania, Italy
- <sup>8</sup> Department of Agricultural Sciences, University of Sassari, Italy
- <sup>9</sup> Institute of Organismic and Molecular Evolution, Johannes Gutenberg University Mainz, Germany
- <sup>10</sup> Research Institute for Biosciences, University of Mons, Belgium
- <sup>11</sup> Biological Sciences and Bioengineering, Sabanci University, Turkey
- <sup>12</sup> State Museum of Natural History Karlsruhe, Germany
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Bumblebees provide an essential ecosystem service in crop pollination. In particular, the bufftailed bumblebee Bombus terrestris is economically important across Europe. This widespread species shows a remarkable phenotypic diversity with nine recognized subspecies. Recently, population genomic studies from the British Isles and Central Asia have shown subspeciesspecific genetic diversity, population structure and signature of selection, while large parts of the existing diversity of the species remain unexplored despite their conspicuous phenotypic variation. Here, we use comparative and population genomics to explore the evolutionary history, current genetic diversity and sub-specific divergence and adaptation across nearly the entire range of the species. While we show that continental subspecies show relatively little differentiation, populations of the subspecies from Sardinia, Corsica, Canary Islands, and the British Isles show a remarkably high level of differentiation, often accompanied by significant phenotypic changes. This high degree of differentiation is surprising in those cases where populations on very large islands formerly shared the same range, but got disconnected during glacial events only short evolutionary times ago. In addition to single nucleotide polymorphisms, we show that structural variations might play a large role in population divergence. Population genomics and signature of selection analyses show that the genetic differences between subspecies are mostly found in coding regions, suggestive of selective forces and local adaptation impacting genes, of which many are transcription factors or have functions related to signalling pathways. Our results, integrated into a phylogenetic context, provide a comprehensive perspective on the evolution and diversification of the important pollinator species Bombus terrestris.

### Marking homologous genetic neural lineages reveals developmental differences in brains of flies and beetles with likely relevance to behavior

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Evolutionary adaptations of brain structure and function are essential for animal survival and emerge during development. Some known divergences seen in brain development are likely relevant for brain function. For instance, adult neurogenesis in mushroom bodies is found in beetles but not in flies and might modify learning behavior. Further, beetle larvae have a partial central complex (CX) while fly larvae lack a visible CX. However, the cellular and genetic mechanisms controlling diversification remain enigmatic. To study brain diversification both, between life stages and between taxa, we have been using homology-directed genome editing to establish transgenic lines that visualize homologous cell groups throughout development. Specifically, we marked all cells expressing the transcription factor retinal homeobox (rx) in the fly Drosophila melanogaster and the beetle Tribolium castaneum. These genetic neural lineages are precise tools to mark and compare the development of homologous neural cells from the embryo to the adult. Our efforts have revealed several intriguing differences of brain development between fly and beetle: We find nine type II neuroblasts in beetle embryos (compared to eight found in flies and grasshoppers) and an increased division activity of these neuroblasts compared to fly embryos. This correlates with the embryonic emergence of a central complex in beetle larvae. In the adult brain, we found a cell cluster in flies, which seems to be absent in beetles. Finally, a change of rx expression in the mushroom bodies likely correlates with differences in adult neurogenesis between fly and beetle. We follow up these hypotheses by characterizing subpopulations of the genetic neural lineage by single cell sequencing and by testing candidate regulator genes and hormone pathways for their role in shifting developmental timing.

### Modelling Biodiversity Decline: Conservation Genomics and Ecological Niche Modelling of *Numenius arquata*

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The rapid loss of global biodiversity is an increasingly urgent issue that threatens life on earth. Anthropogenic activities leave fragmented habitats lacking heterogeneity in landscape causing a decline in species richness. With the high number of threatened species, and many more species not regularly assessed, the methodology for assessing extinction risk needs to be reevaluated to include genetic and genomic analyses in addition to traditional population monitoring. It is this sentiment that has sparked the creation of the BiGFoot (Biodiversity Decline's Genomic Footprint) project. Here, we are investigating the population decline of the threatened Charadriiform species, Numenius arquata, a species which has been continuously in decline due to fragmentation and habitat loss (Johnsgard 1981), to determine the vulnerability to extinction. By incorporating ecological niche modeling and genomic analyses using data from past and present populations, we aim to (i) estimate ancestral population size and point of decline (ii) compare past and present models to determine if the change in suitable habitat for the species coincides with extinct populations (iii) use genetic analyses to compare populations within regions which the model has deemed highly favorable and less favorable. The combination of demographic history reconstruction, population genomic analyses, and ecological niche modeling offers a comprehensive interdisciplinary approach to extinction risk assessment, to better direct conservation efforts.

Johnsgard, P.A. 1981. The plovers, sandpipers and snipes of the world. University of Nebraska Press, Lincoln, U.S.A. and London.

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### Genome-wide DNA methylation profiles from different developmental stages and castes of the harvester ant *Pogonomyrmex californicus*

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The term epigenome refers to the assemblage of all epigenetic changes along the genome, some of these epigenetic modifications could be transfered from one generation to the next. DNA methylation is arguably the most extensive researched epigenetic mechanism demonstrated that variation on DNA methylation patterns, especially cytosine methylation within the CpG context, alters transcription of the methylated gene. Furthermore, DNA methylation has been suggested as a key mechanism in charge of the development of different castes in social insects. Our study described for the first time the methylome of the harvester ant Pogonomyrmex californicus. We compared genome wide DNA methylation patterns between three different life stages (larva, pupae, adult) and two female castes (queens and workers) using two methods: Nanopore sequencing (Ont) and whole genome bisulfide sequencing (WGBS) to deeper understanding of the function of DNA methylation in ants and contribute to elucidate the influence of DNA methylation on the caste specialization. We determined that the methylome of P. californicus possessed low levels of DNA methylation (3% of methylated CpGs) like that has been found in other Hymenoptera species. The highest level of methylation was presented on the gene regions along the genome, and inside the gene region methylation was more polarized on exons than on introns. Promoter region, intergenetic regions and transposable elements present the lower levels of methylation. Developmental stages show distinct methylation patterns which could affirm the role of DNA methylation as a proximate mechanism that regulates gene expression and affect caste specialization in social insects. In addition, our DNA methylation estimate data obtained from Nanopore were as accurate as the ones detected by WGBS. Furthermore, our results support that gene body methylation strengthens gene expression, as we found that DNA methylation of gene bodies increased gene expression levels in *P. californicus*.

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#### Linked selection of insertions and deletions in coding regions of the great tit genome

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Insertions and deletions (indels) are the most frequent structural variations and the second most common type of genetic variation in genomes, with the potential to affect multiple bases within one single mutational event. However, the genetic footprints of selective forces on indel mutations, such as hard sweeps and soft sweeps, remain unknown and challenging due to technical limitations. In this study, we calculated the non-synonymous substitution rate (dN), the synonymous substitution rate (dS), and the ratio of nonsynonymous to synonymous substitutions ( $\omega$ ) in the regions surrounding indels to evaluate their genetic footprints in the great tit genome. Our findings reveal that the regions surrounding indels exhibit low dS values, indicating that mutations in these regions are not random. Furthermore, the strength of selection on these indel surrounding regions not only varies between insertions and deletions, but is also significantly influenced by indel size.

### A chromosome scale reference genome for the Ural owl (*Strix uralensis*) demonstrates the applicability of cell cultures as sources for genomics of endangered species

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While reference genomes are becoming available for many bird species, there are currently only a handful for the family Strigidae, which counts 310 described species. In this study, we generated a high-quality reference genome for the Ural owl (*Strix uralensis*) to support conservation efforts and genomic studies within this family. Using this genome, we analysed candidate genes linked to nocturnal hunting adaptations and feather coloration as well as more broadly gene families that have expanded or contracted within Strigidae. Additionally, we explored the feasibility of using cell cultures as a DNA source for sequencing for species with limited biological samples, a challenge often encountered in conservation genomics. Our research assessed mutation accumulation over multiple cell lines, focusing on the stability and quality of DNA across extended culture. This study will offer valuable resources for research on Strigidae and other avian species and help guide future work on the use of cell cultures for DNA sequencing.

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### Investigating the mystery of PRDM9-independent meiotic recombination pathways in Canids

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Successful recombination is an essential driving force of evolution, being crucial for sexually reproducing organisms to generate functional germ cells and ensure genetic variation in the offspring. In this fine-tuned, yet enigmatic process, one highly conserved gene in particular was found indispensable in most metazoan taxa; PRDM9. Lack of functional PRDM9 in almost all mammalian species typically leads to early meiotic arrest and subsequent infertility, including in humans. However, the family of Canidae evolved to lack functional PRDM9 millions of years ago without suffering these cytotoxic consequences. What mechanism saves canine germ cells from meiotic arrest? Given that there have been rare findings of PRDM9-dependent organisms being able to cope with PRDM9-deficiency, including one human female, it is likely that this mechanism might be even more ancient and conserved than PRDM9 and had a higher evolutionary advantage in Canidae. Consequently, unravelling this pathway could even be a potential answer to claimed "idiopathic" infertility cases in humans, which originate in PRDM9deficiency. In this project I analyze the PRDM9-independent recombination landscape of the domestic dog at the fine-scale, using high-resolution sperm-typing techniques with cuttingedge Nanopore sequencing tools. With this knowledge, we can start understanding what mechanism took over in canines that is able to overcome the typically cytotoxic consequences of lacking PRDM9. These results allow me to unravel the most enigmatic mammalian recombination pathway(s) that are PRDM9-independent - laying the cornerstone in also understanding the underlying reason of PRDM9-absence induced meiotic arrest and infertility in humans.

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### Localised tissue-specific gene expression and gene duplications are important sources of social morph differences in a social bumblebee

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Understanding the expression of multiple behaviourally and morphologically distinct phenotypes from a single genome represents a fundamental topic in evolutionary biology. Central to the complication of expressing phenotypes, which may differ in their optima, is the sharing of largely the same genome, which is predicted to manifest in conflict at the genomic level. This is particularly true for social insects where molecular mechanisms, such as differential gene expression, contribute to observed phenotypic differences between reproductive and non-reproductive morphs. In comparison, other mechanisms, such as tissuespecific expression and gene duplications, have been posited as contributing to social morph differences yet formal investigations are limited. Here, using a combination of transcriptomics for multiple tissues and comparative genomics, we show that in a social bumblebee, the strongest differences in gene expression are found in the spermatheca, an organ previously believed as vestigial in workers but recently shown as functional. In comparison, we find modest expression differences in genes between queens and workers for tissues traditionally investigated for roles in social evolution. Interestingly, such genes display higher tissuespecificity suggesting that while social morphs may express a shared core transcriptome, localised expression profiles may contribute to phenotypic differences. We also find evidence of differential usage of duplicated genes by queens and workers, highlighting structural variants as a contributing factor to morph differences. Collectively, our findings highlight how social insects can utilise tissue-specific gene regulation and structural variants to contribute to differences at the phenotypic level.

### Pathogen non-planktonic phases within the urinary tract impact early infection and resistance evolution

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Treatment of urinary tract infections (UTIs) and the prevention of their recurrence is a pressing global health problem. In a UTI, pathogenic bacteria not only reside in the bladder lumen but also attach to and invade the bladder tissue. Planktonic, attached, and intracellular bacteria face different selection pressures from physiological processes such as micturition, immune response, and antibiotic treatment. Here, we use a mathematical model of the initial phase of infection to unravel the effects of these different selective pressures on the ecological and evolutionary dynamics of UTIs. We explicitly model planktonic bacteria in the bladder lumen, bacteria attached to the bladder wall, and bacteria that have invaded the epithelial cells of the bladder. We find that the presence of non-planktonic bacteria substantially increases the risk of infection establishment and affects evolutionary trajectories leading to resistance during antibiotic treatment. We also show that competitive inoculation with a fast-growing non-pathogenic strain can reduce the pathogen load and increase the efficacy of an antibiotic, but only if the antibiotic is used in moderation. Our study shows that including different compartments is essential to create more realistic models of UTIs, which may help guide new treatment strategies.

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### Effects of the GMO protein Cry1Ab extracted from MON810 Bt maize on the target organism *Ostrinia nubilalis* and the non-target organism *Camponotus maculatus*

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Agriculture is essential for sustaining the global food supply for both human and animal consumption. However, monoculture practices make crops highly vulnerable to pests and diseases, leading to significant yield losses. To combat this, pesticides have been widely used, but their broad-spectrum activity can harm non-target organisms (NTOs). Genetically modified organisms (GMOs) were developed as a targeted approach to protect crop yields, focusing on specific pests while reducing unintended harm. One such development is the use of Cry proteins, derived from the bacterium Bacillus thuringiensis (Bt). These proteins target specific insect groups, including Lepidoptera, a major agricultural pest group, making them a potentially safer alternative to traditional pesticides. Cry proteins bind to receptors in the insect midgut, causing easing of cell contents into the body cavity, ultimately leading to death. Bt maize, a GMO crop that produces Cry proteins, has proven effective in controlling target pests. However, concerns remain about its effects on NTOs, such as pollinators and predators indirectly exposed to the proteins. Although Cry proteins are highly specific, further research is needed to assess potential ecological impacts. The Bt maize variant MON810 produces Cry1Ab, a protein that targets the European corn borer (Ostrinia nubilalis), a lepidopteran pest with significant impact. O. nubilalis is often used as a model organism to study Cry protein efficacy and specificity, but little attention has been given to NTOs exposed by feeding on affected TOs. When O. nubilalis larvae were exposed to MON810 Bt maize material, the mortality was almost three times as high as when exposed to non-Bt maize. On artificial food mixed with juice, similar effects were observed, showing the toxicity of the protein on its TO. Tests with Camponotus maculatus, a predatory ant and NTO, should show no lethal or sublethal effects, due to the absence of Cry1Ab-specific receptors.

#### De Novo Discoveries: Innovative Tools for De Novo Gene Research

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De novo genes emerge from previously non-coding regions of the genome, challenging the traditional view that new genes primarily arise through duplication and adaptation of existing ones. Characterised by their rapid evolution and their novel structural properties or functional roles, de novo genes represent a young area of research. Therefore, the field currently lacks established standards and methodologies, leading to inconsistent terminology and challenges in comparing and reproducing results. We here present novel tools to facilitate de novo gene detection and method annotation with a focus on user-friendly access through both graphical and command-line user interfaces. Our tool, DESWOMAN, detects de novo expressed open reading frames in transcriptomes, validates their de novo status via synteny analysis, and identifies enabling mutations. DeNoFo is a toolkit that provides easy access to a developed annotation format to document methodology of de novo gene research in a standardised and reproducible way, ensuring consistent and reproducible annotations that facilitate effective comparison across studies. These tools improve the reproducibility and comparability of de novo gene studies. Their accessible design supports interdisciplinary collaborations and advances in this rapidly evolving field of research.

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#### **NUMT** expression in ant genomes

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Nuclear mitochondrial pseudogenes (NUMTs) are fragments of mitochondrial sequences that have been integrated into the nuclear genomes of most eukaryotic organisms. Usually, NUMT insertions are not expressed and do not cause obvious phenotype changes. However, they provide a source of genomic variation and, in rare cases, may affect gene expression or even cause disease. To investigate the number of NUMTs in ants, we screened 77 ant genomes available in NCBI and the GAGA project using BLAST. The number of detected NUMTs varied from 4 to 324 per ant genome. For eight species with a high number of NUMTs, we analyzed their potential genomic expression. For this, we performed transcriptome mapping using STAR. Using a conservative approach, we only accepted NUMTs as expressed if they had ≥ 10 substitutions compared to the mtDNA sequence of the analysed ant species. We indexed 674 NUMTs from eight species and identified 16 expressed NUMTS. Eleven NUMTs were expressed in the genomes of Camponotus fellah, three in Monomorium pharaonis, one in Anoplolepis gracilipes, and one in Buniapone amblyops. Nine mitochondrial genes and three tRNAs were involved in NUMT expression. Eight expressed NUMTs were found in introns, four in UTRs, and four in intergenic regions. In most cases, complete or partial transcripts of NUMTs extend to adjacent genomic regions, whereas in one case, only internal fragments of the NUMT were transcribed. We also found two annotated genes located within NUMTs in the genome of C. japonicus and one gene in the genome of C. fellah. Thus, the presence of transcribed NUMTs and their potential to contribute to novel gene formation suggests that, although most NUMTs are evolutionary neutral, insertions in phenotypically relevant regions such as of introns, UTRs and/or long non-coding RNAs may play a role in gene regulation, RNA processing, and overall ant biology.

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### Partition Models Enhance Phylogenetic Inference of Fireflies from Mitochondrial Genomes

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Mitochondrial genomes are widely used in phylogenetic inference due to their biological characteristics, such as relatively high mutation rate and uniparental inheritance, as well as ease of sequencing and costs. Despite their long history of use in phylogenetics, few detailed studies have focused on appropriate models of substitutions. Uniform substitution models, which assume identical evolutionary rates among loci, are commonly applied in mitochondrial phylogenetic analysis to represent sequence evolution. However, high intragenomic evolutionary dynamics have been reported in mitochondrial genomes, with substitution rates found to vary by region. Therefore, uniform models that overlook site heterogeneity are not a realistic representation of mitochondrial evolution, while models with partitioning of data into subsets provide a more accurate depiction. To assess different partitioning models in mitochondrial phylogenetics, we conducted a phylogenetic analysis in RevBayes on a dataset of 104 firefly (family Lampyridae) specimens, including 22 newly collected samples, focusing on 13 protein-coding genes and two ribosomal RNA genes. We estimated and compared the timecalibrated phylogeny under four partition models (uniform, by codon position, by gene, and by gene and codon position) combined with two clock models (strict clock and relaxed clock). We found the choice of clock model resulted in distinct tree topologies while the choice of partition model has little impact. Comparison of partition models showed significant difference in evolutionary rates of the two ribosomal RNA genes and the 13 protein-coding genes in mitochondrial genomes, as well as higher evolutionary rates at the third codon position in protein-coding genes, confirming site heterogeneity in mitochondrial evolution. Additionally, partition models influence divergence time estimates, with more complex partitioning resulting in an older estimate for the divergence of crown fireflies. Our results indicate that more complex partition models are required for robust estimation of divergence times from mitochondrial genomes.

### Mapping the Uncharted Sequence Space: A k-mer Powered, Alignment-Free Journey through Protein Sequence Space

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Understanding distances and distribution of protein families in protein sequence space is crucial for both molecular evolution and protein engineering. We explored the sequence space between three distinct types of protein sequences: de novo emerged proteins, random sequences, and conserved protein families, using an alignment-free, k-mer-based approach based on the novel computational tool SHARK. By calculating k-mer frequencies and measuring distances between those k-mers, we effectively capture the distributional and compositional differences between sequences in protein sequence space where alignmentbased approaches have failed before. Our approach reveals distinctions in sequence diversity and conservation, offering a new perspective on the very fabric of protein sequence space and distances between different protein families, random sequences and evolutionary young de novo proteins. By identifying specific regions within sequence space associated with emergent functionality, our approach opens additionally new avenues for the design of proteins with desired properties, leveraging the principles of natural evolution. Our k-mer-based, alignmentfree framework serves as a powerful approach for both deciphering evolutionary origins and advancing protein design, enhancing our understanding of how sequence diversity translates into functions and structures.

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#### Meta-analysis of prosocial behaviour in nonhuman mammals and birds

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Prosocial behavior is defined as any behavior that is intended to benefit another individual. This behavior does not need to be costly. Results of individual studies are heterogenous, obscuring major effects. We conducted a phylogenetically controlled meta-analysis in order to investigate how much variation in non-human mammalian and avian prosocial behavior can be explained by different experimental approaches and prosocial-choice paradigms. We examined if variation in prosocial behavior can be predicted by social organization and care systems, and their associated social tolerance. We calculated Hedge's g as the effect size to compare the outcome of conditions for the actor and the recipient: 1) selfless (0/1) vs spiteful (0/0), 2) mutualistic (1/1) vs selfless (0/1), 3) mutualistic (1/1) vs selfish (1/0), 4) selfish (1/0) vs selfless (0/1), and 5) mutualistic (1/1) vs spiteful (0/0). We compared the test condition, i.e. partner present, to different controls conditions, i.e. partner absent or random chance. We classified the experimental design according to the techniques used in the experiment (prosocial choice task: bar-pulling, symbol/token; giving assistance task: helping, token exchange, or release paradigm). We conducted meta-regressions to assess the influence of allomaternal care, the care system (cooperative breeding, communal breeding, group-living) on the effect sizes. The meta-analysis included over 40 studies, 160 test effect sizes and 210 control effect sizes to investigate which factors may explain the evolution of prosocial behavior. Preliminary results indicate that the care system did not significant affect the effect sizes for the 1/1 vs 1/0 and the 1/1 vs 0/0 comparisons, and these results did not support the cooperative breeding hypothesis for prosociality. However, categories of care system are rather broad categorization of allomaternal care. Hence, additional analyses, including the amount of allomaternal care, are now indicated.

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#### Evolutionary genome dynamics in asexual mite species

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Sexual reproduction is often viewed as essential for species evolution and long-term persistence. Yet, the existence of ancient asexual organisms, such as certain oribatid mites, challenges this view. How these organisms cope with challenges associated with asexuality remains unclear. Here, we generated high-quality genome assemblies for eleven oribatid mites, spanning four independent transitions to asexuality and sexual relatives, to examine genome dynamics associated with asexuality. Our analyses confirm that asexual mites do not exhibit increased mutation loads, indicating that selection remains as effective as in sexual species. Moreover, the genomes of asexual species are not enriched in rearrangements and contain fewer transposable elements compared to their sexual counterparts, possibly due to their high effective population sizes (Ne). Using PacBio HiFi population re-sequencing, we explore whether Ne differs between sexual and asexual species and analyze segregation dynamics of short- and largescale genomic rearrangements at the population level. By combining comparative and population genomics, this project will provide insights into how ancient asexuals escape the predicted evolutionary dead-end and how the release from the constrains of sex might offer evolutionary advantages.

### Avian Genomics: Transposable Element Dynamics and Their Regulatory Potential in the Great Tit

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Transposable Elements (TEs), also known as "jumping genes" or repeats, are genomic features capable of replicating themselves and moving throughout a host genome. As ambiguous genomic features, transposable elements are infamous genomic parasites but have been increasingly rebranded as sources of genomic novelty. Though studies on beneficial TE insertions have historically focused on functional proteins, a growing body of research has highlighted the regulatory potential of TEs, suggesting that TEs may provide an important and sometimes beneficial source of epigenetic novelty to their hosts. TEs are less common in birds than other vertebrates, and as such have drawn less attention in class Aves. TEs are nonetheless assumed to be active in birds and, in the great tit—a key model for avian genomics—are linked to DNA methylation and gene regulation. Here, we evaluate TEs of great tits as a potential source of beneficial epigenetic novelty. We begin by characterizing the repeats of great tits and assessing their potential to mobilize. It was found that great tits possess a repeat landscape resembling other birds with most repeats belonging to retrotransposons, such as Chicken Repeat 1 (CR1) and Endogenous Retrovirus (ERV) elements. We confirmed that great tits have a dearth of active repeats, with all identified repeats having ancient insertions. We find however that many ancient TE insertions retain high levels of GC and CpG content relative to their locations of insertions, despite a general trend for repeats to lose GC bases and CG dinucleotides overtime. Furthermore, several of these conserved repeats were differentially methylated between blood and brain tissue, suggesting that they are potentially important to gene regulation. These findings demonstrate that TEs are a source of epigenetic novelty in great tits, and call for further analysis of avian TEs in epigenetic context.

#### Host specialization defines the emergence of new fungal plant pathogen populations

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Host specialization is considered the strongest driver of pathogen evolution. To successfully infect, colonize and complete the life cycle, plant pathogens are under constant selective pressures imposed by hosts, leading to adaptative genomic evolution and possibly new pathogen species or lineages radiation. Implementing population and comparative genomics approaches, we identified evolutionary signatures of divergent host specialization in distinct lineages of the fungal pathogen *Zymoseptoria tritici*, a major disease causing-agent of wheat. Unique collections of Z. tritici were isolated from wild (Aegilops spp.) and domesticated (Triticum aestivum) host grasses in the Middle East, and whole-genome sequencing was performed in a subset of isolates from each collection. We observed distinct population structure between the two host-diverging pathogen collections and particular genomic features in the Aegilops-infecting isolates that may have shaped their evolutionary history. Phylogenomic analyses indicated that A. cylindrica and A. tauchii-infecting populations of Z. tritici form separate clusters when compared to worldwide collections of Z. tritici and to closelyrelated species, possibly reflecting incipient speciation driven by divergent host specialization. Using infection experiments, we confirm that Z. tritici isolates collected from Aegilops spp. only infect their respective host species and not T. aestivum. Population genomic analyses and demographic inference furthermore allowed us to detect signatures of recent selection and show that divergence of these host-specific lineages likely coincided with wheat domestication. At last, we confirm a virulence-related role for one candidate effector located in a selective sweep region of the A. cylindrica-infecting pathogen. Taken together, our findings highlight the interplay between agricultural and wild hosts on the evolution of fungal plant pathogens and illustrate host specialization as a possible route of rapid pathogen emergence.

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## Stability versus flexibility - knocking down canalization regulating mechanisms in *Cardiocondyla obscurior*

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In ant colonies the queen caste is responsible for reproduction, while the sterile worker caste maintains and defends the colony. This reproductive division of labour between specialized individuals contributing to the survival and functionality of the whole is a hallmark of superorganismality. In analogy to the germline of multicellular organisms, the queens of superorganisms are expected to be better protected against environmental variation to secure the reproductive capacity of the colony, leading to more stable phenotypes in queens. In the somatic worker caste, selection for phenotypic robustness should be relaxed, rendering workers more susceptible to environmental changes. To explore the molecular and genetic mechanisms regulating phenotypic robustness and canalization in the castes of ants, we employ developmental RNA interference (RNAi) in Cardiocondyla obscurior. The Myrmicine ant C.obscurior is as an exceptional model for studying superorganismality and caste evolution, as workers have entirely lost their ovaries and queens and workers can be distinguished already during the first larval instar. By experimental knockdowns of genes involved in phenotypic canalization (e.g. Hsp90) or caste determination (e.g. freja), we will be able to reveal caste-specific modulations of phenotypic canalization, providing insights into the molecular foundations of superorganismality.

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#### Wolbachia endosymbionts in bees - protectors, nurturers, or parasites?

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Many insect species harbour intracellular symbiotic bacteria that are passed on from mothers to offspring. These symbionts employ various strategies that enhance their proliferation, which may impact the host's fitness in multiple ways. Wolbachia (Alphaproteobacteria: Rickettsiales) is a common endosymbiont of arthropods but is especially frequent in bees (Hymenoptera: Anthophila). The reason for its success in this particular group of insects is unclear. We here explore three alternative, not mutually exclusive roles Wolbachia may play in bees. First, we determined if Wolbachia may block viral infections in bees, as has been described for other hosts. We sampled hundreds of bee individuals from three common species for which we characterized the virome, and determined viral and Wolbachia loads. We found no conclusive evidence for Wolbachia influencing viral titres in bees. Second, we used comparative genomics across a wide range of Wolbachia strains to identify potential signatures for nutritional supplementation by the symbiont. This revealed that unlike in most other hosts, Wolbachia in bees is able to synthesize biotin, which may be supplemented to the host. This ability has at least two independent origins in bee Wolbachia and is linked to MGEs in the Wolbachia genome. Third, we characterised the Wolbachia genes known to cause reproductive manipulations in other hosts and found a large diversity and high copy number of these loci in bee Wolbachia. Our data suggest that Wolbachia likely has various effects in bees. We outline avenues for future work and discuss limitations in studying endosymbionts of non model organisms.

#### A comparative study of neural differentiation in primates

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Advances in transcriptomics and comparable cell culture conditions across species allow us to study recent evolution not only on gene sequence, but now also on gene expression level in a specific cellular context. Leveraging dynamic processes across the primate phylogeny can inform on the molecular basis of phenotypes and human disease, because conserved gene expression patterns are expected to reflect functional importance. Here, we aim to strengthen this hypothesis by investigating a dynamic process in a cross-species differentiation context. We investigated early neural differentiation in two great apes, human and gorilla and an Old World monkey, cynomolgus macaque, in multiple biological replicates across six timepoints using single cell RNA-sequencing. After aligning temporal expression trajectories across species to assure comparability, we identified a set of genes that is constantly upregulated during the cell state transition from induced pluripotent stem cells to neural precursor cells in all three species. We characterized the genes with conserved expression upregulation and found that a substantial fraction is associated with relevant differentiation processes. Moreover, the monotonically and consistently upregulated genes show higher sequence conservation across primates and a higher probability of being mutation intolerant in humans than genes upregulated only in humans. This set is furthermore enriched in transcriptional regulators associated with known neurodevelopmental diseases, suggesting high functional relevance for the process of neural differentiation. In summary, we show that a deeper understanding of the link between regulatory conservation and functional relevance can strengthen the confidence when addressing less commonly investigated cellular processes and help to prioritize particular dysregulated genes in the context of disease.

## Sources of genetic variation and diversity of three maternally inherited genetic elements in a songbird

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Not all cells contain the same set of genetic information. Certain DNA sequences are present only in the germline and absent from any somatic tissue. This programmed DNA elimination usually occurs during embryonic development and can lead to the loss of an entire chromosome, such as the songbird germline-restricted chromosome (GRC) found in Passeriformes (~6000 species). The GRC typically occurs as a single copy in males, where it is eliminated during spermatogenesis, meaning it is inherited only through the matriline. In females, two identical copies of the GRC are present, ensuring its stable inheritance and indicating a lack of recombination between different GRC haplotypes. Despite its stable inheritance and indispensable nature, the evolutionary dynamics of the GRC remain understudied, particularly in comparison to other strictly maternally inherited elements, such as the W chromosome and mitochondrial DNA (mtDNA). Recently, our group found extremely low genetic diversity in the zebra finch (*Taeniopygia guttata*) GRC using SNP-based analyses. In this project, I will further explore the genetic diversity of the zebra finch GRC by focusing on insertion and deletion of genes, as well as structural and copy number variations across different captive populations of zebra finches. Additionally, I will compare genetic diversity estimates and phylogenetic relationships between the GRC, the W chromosome and mtDNA to better understand the evolution and adaptation of the GRC in the context of Hill-Robertson interference. By comparing the population genetics of typically maternally inherited genetic elements, this project aims to provide novel insights into the sources of genetic variation and the evolutionary significance of the GRC in songbirds.

#### Inter- and intraspecific genetic diversity in worldwide Mammals along Latitudes

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The latitudinal gradient in biodiversity is a long-observed phenomenon, where many components of global biodiversity, including species richness, phylogenetic diversity, and functional diversity, decrease from the Equator to the Poles. Though nuclear genetic diversity is an important component of biodiversity that underlies species' adaptive potential, whether it also varies latitudinally with other forms of biodiversity is unclear. Additionally, unlike other forms of biodiversity which are typically measured at the species level, genetic diversity has both within- and between-species components that may each be predicted to covary with latitude. Within-species genetic diversity patterns emerge in part from population-level microevolutionary processes, while spatial patterns of between-species genetic diversity are products of species-level variation and species turnover. Together, within- and betweenpatterns of genetic diversity can contribute to a more comprehensive understanding of the evolutionary and ecological processes maintaining global biodiversity patterns. The increasing availability of publicly accessible genetic data worldwide allows us to investigate whether a latitudinal gradient also exists in genetic diversity. Here, we use a unique dataset of 67,000 individual genotypes sampled from 112 species and 1697 local populations to investigate latitudinal patterns of neutral nuclear genetic diversity in mammals. Preliminary results suggest that there is no evident latitudinal gradient in genetic diversity either within or across species. We will discuss the lack of genetic latitudinal gradient in light of theories underlying the species richness gradient and their predictions regarding population-level processes.

#### Of Flies and Men - The Evolution of Gene Regulation

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Phenotypic diversity is often caused by differences in 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. In our group, 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. 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.

#### The nematodes *Panagrolaimus*: a new model for cryptobiosis in animals

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Panagrolaimidae is a widely distributed family of free-living nematodes and includes over 300 documented strains on NCBI Taxonomy. Among these, Panagrolaimus species have been shown to survive desiccation, and some Panagrolaimus species can survive freezing by undergoing cryptobiosis, a state of slowed metabolism in response to extreme environments. In addition, these worms have various modes of reproduction including hermaphroditism, gonochorism, and a transition to strict parthenogenesis in the genus Panagrolaimus. So far, genomic resources for this family are scarce, with only one chromosome-level assembly for Turbatrix aceti, and some highly fragmented assemblies. To gain deeper insight into the evolution of Panagrolaimidae from a genomic perspective, we generated 8 chromosome-level and additionally 12 highly contiguous assemblies for Panagrellus, Propanagrolaimus and Panagrolaimus species, and a phased assembly for a parthenogenetic Panagrolaimus. Chromosome-level assemblies reveal 5 chromosomes (2n=10) in most Panagrolaimidae species, and 4 chromosomes (2n=8) in one branch of Panagrolaimus resulting from a chromosome fusion. Gene synteny shows large inter and intraspecies rearrangements in Panagrolaimus that are not observed amongst other Panagrolaimidae genera, and may be linked to DNA strand breaks and repair during cryptobiosis. Further investigations into the 20 genome assemblies show larger integration of horizontal gene transfer among *Panagrolaimus*, and increased genome sizes through the evolution of Panagrolaimus and the acquisition of cryobiosis. Our analysis yields insights into the previously understudied *Panagrolaimus* genus and highlights its potential as a new model for cryptobiosis.

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#### Molecular Signatures of Healthy Ageing in Long-Lived Rodents

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In our project, we are investigating the molecular signatures of healthy ageing in long-lived rodents, focusing particularly on the naked mole-rat (NMR) and other species that exhibit significant lifespan variations. Traditional ageing research has primarily centred on short-lived organisms; however, our study aims to elucidate the genomic and transcriptomic factors that contribute to longevity by leveraging data from 45 high-quality rodent genome assemblies. Preliminary analyses reveal several key findings. We generally observe increased evolutionary rates in longer-lived species, likely due to relaxed selection stemming from low effective population sizes. Notably, we have detected several genes whose selection strength correlates positively with lifespan, underscoring their importance for healthy ageing. Specifically, genes that are over-expressed in older individuals show a lack of a selection shadow in long-lived species compared to short-lived rodents, suggesting efficient selection on genes critical for survival in advanced age. Additionally, we find indications of greater DNA methylation levels in long-lived species, which may reflect epigenetic mechanisms that promote healthy ageing. Furthermore, we anticipate observing less variation in transcription factor binding sites in longlived species, which could facilitate more stable transcriptional regulation throughout life. Within our research project, we will also generate novel transcriptomic data for the NMR, Damaraland mole-rat (DMR), and blind mole-rat (BMR), focusing on various tissues across different ages. By employing deep learning techniques, we are developing neural networks to predict individual ages and maximum lifespan based on genomic and transcriptomic features, thereby enhancing our understanding of the most informative traits associated with longevity. Ultimately, this project seeks to identify universal genomic footprints of longevity that extend beyond protein-coding regions, illuminating critical pathways involved in the hallmarks of ageing, including nutrient sensing and mitochondrial function, as well as previously underappreciated pathways. Our findings could significantly impact future research in gerontology and inform therapeutic strategies to promote healthy ageing in humans.

### Neighborhood matters: How local adaptation shapes metabolic interactions in soil bacterial communities

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Bacteria are remarkably versatile organisms that thrive in diverse environments, from deep oceans to arid deserts. Their ability to adapt is crucial for survival and drives population evolution. Interestingly, many free-living bacteria, called auxotrophs, cannot produce essential metabolites and rely on their environment or other microbial partners for nutrients through obligate cross-feeding. This dependency may promote local adaptation among bacterial partners over evolutionary timescales. Most studies on bacterial local adaptation emphasize abiotic factors such as temperature and pH, with less exploration of biotic interactions. We addressed the following question: Are auxotrophic bacteria in natural communities better adapted to their local partners? To examine this, we employed a system to evaluate the metabolic exchange between amino acid auxotrophs and metabolically autonomous prototrophs. We measured local adaptation by evaluating the fitness of auxotrophic strains in sympatric (same community) versus allopatric (different community) environments, leveraging a collection of over 1,000 bacterial strains from 27 soil microbial communities. Pairwise coculture experiments on agar plates showed that auxotrophs grew significantly better with sympatric prototrophs than allopatric prototrophs. This pattern was further corroborated by observations that auxotrophs exposed to supernatants from a community of 15 sympatric prototrophs grew significantly better than those exposed to supernatants from allopatric communities. To further examine the role of interactions in the communities of donor genotypes, we compared auxotroph growth in reconstituted supernatants (mixtures of singlestrain supernatants) to community supernatants (co-cultured prototrophs). This experiment showed that supernatants from the complete sympatric community enhanced the growth of auxotrophs, whereas the reconstituted sympatric supernatant did not, indicating that auxotrophic strains adapted to the community as a whole. Our findings demonstrate that auxotrophs are locally adapted to their community partners, relying on them for necessary metabolites and highlighting the importance of local interactions in maintaining natural bacterial communities.

#### A key innovation in the evolution of Lepidoptera

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In modern lineages of the Lepidoptera, larvae consume green plants as their main food. To avoid photoxicity of ingested chlorophyll, modern lineages have evolved polycalins by domain duplication of lipocalin subunits and further gene duplication. These polycalins sequester ingested chlorophyll and other tetrapyrroles, keeping them in the anoxic midgut until they are excreted in the feces. When the polycalin genes of the cotton leafworm are knocked out using CRISPR/Cas9, larvae consuming green plants die in the light, but not in the dark. Knockout larvae survive on artificial diet lacking chlorophyll in the light and the dark, but if tetrapyrroles are added to the diet, they die in the light, but not in the dark. In the knockout larvae, tetrapyrroles are not trapped in the midgut lumen; instead they enter the hemocoel where oxygen is abundant. We hypothesize that singlet oxygen generated by irradiation of the tetrapyrroles causes cellular damage in the hemocoel, mainly by the chain reaction of lipid peroxidation, that can lead to death. So far as we can determine, the genomes of the most primitive Lepidoptera encode single-domain lipocalins but not polycalins. We can also detect single-domain lipocalins, but not polycalins, in genomes of the sister group, Trichoptera. We propose that domain duplication of heme-binding lipocalins was the key innovation enabling lepidopteran larvae to consume large quantities of green plant tissue. This exerted selective pressure on plants to evolve different types of chemical defenses, giving rise to the coevolutionary arms race that fueled the diversification of lepidoptera and angiosperms.

#### Phenotypic selection on heatwave responses of Lymnaea stagnalis snails

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The increasing intensity of global warming challenges the natural population. For instance, global warming results in an increase in extreme weather events, such as summer heat waves, that disrupt the natural processes of organisms. Our study investigates phenotypic selection on organisms' responses to heatwaves. Specifically, we examined (1) how responses of juvenile Lymnaea stagnalis snails to heatwaves vary among individuals, (2) if and how this variation reflects fitness variation among them, and (3) if heatwave-mediated selection is modified by ecological variation (food availability). We exposed ~500 snails (initial shell length: 6-7 mm) to a one-week-long experimental heatwave (27°C) followed by four weeks of maintenance at a benign temperature (17°C). We kept the snails under two different resource availability levels (ad libitum food supply, reduced food supply) and measured their performance as growth rate (an important fitness component influencing reproductive potential after reaching maturity). We measured snail shell length at the beginning of the heatwave and at the end of the heatwave to quantify snail performance during the heatwave (i.e., heatwave response) and also three weeks post-heatwave to understand how the snails recovered after the heatwave and the final size they reached (fitness proxy). Snails enhanced their growth rate during the heatwave and this effect was stronger under ad libitum feeding compared to reduced feeding. The growth rate reduced to a normal level after the heatwave. Selection gradient analysis showed stabilizing selection for snail heatwave responses (i.e., snails with intermediate growth rates reached a higher final size than snails with slow or high growth rates). This was visible in both food treatments. To understand if selection on heatwave responses varies among stages in snail life history, we have also conducted a similar experiment with adult L. stagnalis. In that study, we measured changes in snail growth rate, immune function, and reproductive output to understand heatwave responses and their fitness consequences. The results of that study should be available by the time of the conference.

## Young sex chromosomes in cichlids show rapid evolution of sex-biased genes according to sex determining system

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Sex chromosome turnovers and transitions in sex-determining systems occur frequently in teleost fish, with cichlids exhibiting one of the highest rate of sex chromosome turnover observed to date. The driving mechanism and impact of sex chromosome turnovers and sexdetermining system transitions however remain largely unclear. Sexual antagonism is thought to play a substantial role in the emergences of both sex-biased gene expression and novel sex chromosomes, though experimental evidence has been scarce. In this study, we examined three sex chromosomes in XY and ZW sex-determining systems in Lake Tanganyika cichlid fish species that diverged less than 4 million years ago. All sex chromosomes were enriched in sex-biased genes, with the sex chromosomes of ZW species showing feminization in total sex-biased genes, and an increase in female-biased expression and/or decrease of malebiased expression depending on the tissue investigated. XY sex chromosomes likewise showed masculinization in total male-biased genes, but not in the intensity of sex-biased expression. A large fraction of sex-biased genes show signals of adaptive evolution, with a stronger signal in females than in males in somatic tissue. We conclude that there is some support for sexual antagonism driving sex chromosome differentiation, and suggest the direction sex-bias evolves in young sex chromosomes is influenced by the sex-determining system.

#### **Evolution of repeat transposable elements in bee genomes**

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Bees are essential pollinators and play a crucial role in sustaining ecosystems. With over 20,000 species, they exhibit remarkable diversity. More than 200 species have their genomes sequenced and hence allow to track the evolution of phenotypic traits. Repetitive elements, including transposable elements (TEs), are major drivers of genome (phenotypic) evolution, contributing to genome expansion, restructuring, and genomic diversity. Despite their importance, the evolutionary dynamics of repeats across the vast diversity of bee species remain largely understudied. In this study, we conducted a comprehensive scan of repeats across the genomes of >100 bee species. We performed an individual repeat annotation and quantification using the Earlgrey pipeline. By comparing TE content, composition and divergence, we identified ancient and recent TE burst activities. Integrated into a phylogeny, we estimated the timing of major repeat amplification events, revealing unique divergence patterns in bee genomes. Phylogenetic analyses based on repetitive elements uncovered distinct evolutionary trajectories, emphasizing the strong role of repeats in shaping genomic variation across lineages. Further, explored the relationship between genome size and repeat content as well as the genomic location of TEs, demonstrating that repetitive elements are a key driver of genome expansion and a significant factor in the evolutionary processes of bees. From TE annotation we also built a comprehensive set of bee TEs, suitable for a more generic annotation and comparison.

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#### Age-dependent regulation of gene expression from cold to heat in a desert living bird

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One of the key challenges for endotherms is keeping body temperature constant when exposed to extreme temperature fluctuations. This is especially true for large vertebrates that exhibit large changes in body size and shape during development, which alters their sensitivity to hot and cold temperatures. However, the molecular mechanisms involved in thermoregulation during growth are poorly known. Here, we investigate the genetic underpinnings of thermoregulation in juvenile ostriches (*Struthio camelus*), an endotherm native to environments with large temperature fluctuations, and with the largest change in body size over life stages in birds. For this, we compare gene expression of 1-week and 8-week old juveniles under manipulated temperature conditions, and 12-month old ostriches under natural conditions. Furthermore, we identify genes under selection from three ostrich subspecies living in environments with different temperature regimes to validate the evolutionary importance of genes involved in thermoregulation. The results will shed light on the challenges and conflicts that shape the evolution of adaptive thermoregulation in animals living in environments with pronounced temperature fluctuations.

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### Plant terrestrialization: a phylogenomic and comparative genomic perspective

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Recent developments in omics techniques provide an unprecedented opportunity to gain a deeper understanding of key evolutionary events. I will illustrate this with the origin of land plants. To decipher the evolutionary processes that led to the emergence of land flora, a focus on their closest algal relatives, the streptophyte algae, is essential. Phylogenomics has recently established a robust evolutionary framework for the backbone of streptophytes and delving into the diversity of the various algal lineages is shedding light into cryptic diversity, the origin of multicellularity, and the colonization of terrestrial habitats. Comparative genomics identified expanded genes for signaling cascades, environmental response, and multicellular growth in the immediate algal ancestor of land plants. Gene family analyses showed most phytohormone genes predate streptophytes, but key enzymes are often absent, suggesting stepwise increase of metabolic pathway complexity and in some cases alternative (unknown) metabolic routes. Coexpression analysis of transcriptomic data suggests deep evolutionary roots of the molecular chassis that balances environmental response and growth modulation across more than 600 million years of streptophyte evolution, before plants made their first steps on land. Proteomic and metabolomic characterization of lipid droplets -key for stress response in algae- suggest an evolutionary link between stress response and seed formation. Overall, the integration of various omics techniques is providing a deeper understanding about the origin and evolution of land plants.

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## DNA barcoding of passerine birds at an ornithological crossroad reveals significant East-West genetic lineage divergence

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Exploring genetic diversity is essential for precise species delimitation, especially within taxonomically complex groups like passerine birds. Traditional morphological methods often fail to resolve species boundaries; however, DNA barcoding, particularly through the mitochondrial cytochrome c oxidase subunit I (COI) gene, provides a powerful alternative method for accurate species identification. This study establishes a comprehensive DNA barcode library for Iranian passerine birds, analyzing 537 COI sequences from 94 species across 23 families and 53 genera. We observed a pronounced barcode gap, with average intraspecific divergence at 0.4% and interspecific divergence at 18.6%. Notable intraspecific variation emerged in the Persian nuthatch (Sitta tephronota) and the Lesser whitethroat (Curruca curruca), while the goldfinch (Carduelis carduelis) showed limited genetic differentiation despite marked morphological distinctions. Phylogenetic analysis revealed significant east-west genetic splits in C. curruca and S. tephronota, reflecting Iran's geographic and zoogeographic boundaries. These findings demonstrate the effectiveness of DNA barcoding in elucidating biogeographic patterns, emphasizing Iran's key role as an ornithological crossroads for avian biodiversity. Moreover, our results suggest that much of the genetic variation in the COI gene arises from synonymous mutations, highlighting the role of purifying selection in shaping mtDNA diversity across species.

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#### AnchST - An Annotation-free Synteny Computation and Analysis Toolbox

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We present a novel approach for the computation of synteny anchors based on genomic sequences alone. First results show that our annotation-free approach can be a useful alternative both in terms of computational performance and feasability as well as quality of the synteny anchors. As first described in Käther et al. (2023), we use k-mer statistics as a proxy for the potential of genomic subsequences to serve as synteny anchors and have implemented a complete pipeline to compute respective pairwise syntenic regions for sets of up to several hundred insect species. We will present the web service (http://anchored.bioinf.unileipzig.de:8080) at which these synteny anchors are provided in formats which can directly be used to infer orthology relationships for micro- and macrosyntenic analysis. For instance, they can be used to compute colinear chains of syntenic elements with established tools like MCScanX. Respective application examples like basic orthology inference for high copy number elements and genomic phylostratigraphy will be shown.

Käther, K., Lemke, S., & Stadler, P. F. (2023). Annotation-free identification of potential synteny anchors. In International Work-Conference on Bioinformatics and Biomedical Engineering (pp. 217-230). Cham: Springer Nature Switzerland.

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#### Genomic erosion footprint in insects under extreme decline and isolation

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Global biodiversity is declining critically. Studies in Germany suggest a 70-75% decline in flying insects even in protected areas. Such rapid collapse may pose stark challenges for species and populations due to increases in inbreeding and genetic drift, decreases in genetic diversity and inefficacy of selection. Understanding the genomic processes underlying population declines and extreme isolation is essential to determine genomic erosion in, and assess health of a population, and thereby guide conservation efforts. However, most research focused on vertebrate species, largely overlooking the vast amount of biodiversity: insects and other invertebrates. A high diversity of different life histories gives rise to the expectation that species and populations are impacted or react differently to decline or additional factors such as historical (anthropogenic) influences, bottlenecks, historically small population sizes or longterm geographic isolation. Understanding the genomic dynamics and effects in non-model species is crucial for developing more effective conservation strategies and effective monitoring. Here we take advantage of advancements of sequencing technologies, including museomics, to start to gain knowledge of the genomic footprint of biodiversity decline in nonmodel or non-vertebrate species, including two beetles, a bush cricket, a butterfly, a damselfly and a bee, representing the large insect orders. These species are examples of extreme decline and/or extreme isolation. Population genomics and museomics paired with novel genome assemblies will enable us to uncover the extreme-case genomic signatures of biodiversity decline. This will improve our knowledge for large parts of biodiversity.

# A Juvenile Tale: Heatwave responses and their Evolutionary potential *in Lymnaea* stagnalis

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As climate change accelerates, understanding its effects on organisms is crucial. We conducted two experiments to investigate how juvenile Lymnaea stagnalis snails respond to heatwaves and if those responses show evolutionary potential. In the first experiment, we tested (1) how heatwaves affect juvenile snail growth rate and immune activity, and (2) whether these responses depend on food availability. We exposed the snails to a benign temperature (17°C) and a heatwave temperature (27°C) under three different levels of food availability: unlimited, reduced, and fasting. Results showed that, with ad libitum food, heatwave enhanced snail growth rate, followed by a slight post-heatwave decline. This suggests initial benefits of high temperature followed by costs afterwards. Interestingly, immune activity was not affected by temperature but peaked in snails with limited food, suggesting a strategic reallocation of resources as a response to resource scarcity. Based on these results, in the second experiment, we focused on growth rather than immune activity. We examined if snails show genetic variation (i.e., evolutionary potential) in their heatwave responses by comparing the performance of 12 inbred snail lines in a similar setup as in experiment 1. Snail lines differed in growth rates but not in relation to temperature (no temperature-by-line interaction that would reflect genetic variation in heatwave responses). This finding suggests that snails can benefit from high temperatures for a short time period if external resources are abundant, but the evolutionary potential of heatwave responses of juvenile Lymnaea stagnalis snails is low.

#### **DomRates-Seq: Tracing the Paths of Modular Evolution**

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Protein evolution is central to molecular adaptation and largely characterised by modular rearrangements of domains, the evolutionary and structural building blocks of proteins. Genetic events underlying protein rearrangements are relatively rare compared to changes of aminoacids. Therefore, these events can be used to characterise and reconstruct major events of molecular adaptation by comparing large data sets of proteomes. Here we determine, at unprecedented completeness, the rates of fusion, fission, emergence and loss of domains in five eukaryotic clades (monocots, eudicots, fungi, insects, vertebrates). By characterising rearrangements that were previously considered "ambiguous" or "complex" we raise the fraction of resolved rearrangement events from previously ca. 60% to around 92%. We exemplify our methods by analysing the evolutionary histories of protein rearrangements in (i) the extracellular matrix, (ii) innate immunity across Eukaryota, Metazoa, and Vertebrata, and (iii) Toll-Like-Receptors in the innate immune system of Eukaryota. In all three cases we can find hot-spots of rearrangement events in their phylogeny which can be (i) related with major events of adaptation and (ii) which follow the emergence of new domains which become integrated into existing arrangements. Our results demonstrate that, akin to the change at the level of amino acids, domain rearrangements follow a clock-like dynamic which can be well quantified and supports the concept of evolutionary tinkering.

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Transcriptomic evidence for decoupling of reproductive and non-reproductive roles in the eusocial halictid bee *Lasioglossum marginatum* provides insights into the Ovarian Ground Plan Hypothesis as explanation for the evolution of eusociality

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Eusociality is a major innovation characterized by the reproductive division of labour between females that reproduce (queens) and females that do not reproduce but who take care of the brood (workers). The ovarian ground plan hypothesis (OGPH) has been proposed as a possible evo-devo explanation for the evolution of eusociality. According to the OGPH, maternal care in the solitary ancestor can evolve into sibling care of social descendants through the cooption of brood care by one female phenotype and the concomitant emergence of the an exclusively ovipositing (reproductive) female phenotype. That is, ovipositing and brood care, coupled in the solitary ancestor, are decoupled in social descendants, where oviposition and brood care are undertaken by queens and workers, respectively. Lasioglossum marginatum is a perennial eusocial halictid bee species in which young reproductive females (foundresses) emerge in spring and dig a nest in which they construct brood cells that they fill with pollen and nectar, though they only later oviposit in those cells in summer. Foundresses in spring are therefore functionally more similar to workers than to queens. In summer, then, foundresses are functionally similar to 2-, 3- and 4year-old queens that focus on egg-laying (reproduction). We formulate the following predictions: 1) workers and queens have different transcriptomes (indicating the decoupling of oviposition from brood care); 2) spring foundresses have transcriptomes more similar to those of workers than to those of queens (indicating their functional equivalence to workers). Using transcriptome data from the brain and fat bodies of three female phenotypes (workers, spring foundresses, and queens), we found that the transcriptomes of foundresses are, as predicted, more similar to those of workers than to those of queens. We discuss the implications of these findings for the OGPH in informing on the evolution of eusociality.

## Phenotypic and transcriptomic profiling of *Alectra vogelii* parasitizing cowpea and groundnut

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Alectra vogelii Benth. (Orobanchaceae) is an obligate hemiparasitic weed with relatively wide host range and distribution in the tropics. Despite its economic impact in agricultural landscapes, little effort has been invested in pest management and fundamental research. We aimed at providing the community with the first publicly available large-scale dataset of A. vogelii by generating development-wide transcriptome data of the parasite growing on cowpea (Vigna unguiculata) and groundnut (Arachis hypogaea). A regression-based approach was used to cluster significant gene expression profile differences, which we coupled with selected pairwise comparisons to unveil the parasite's behaviour upon infection of two distinct hosts. Our results highlighted major differences at both phenotypic and transcriptome levels, all of which reflecting on a local host preference for cowpea rather than groundnut. A. vogelii growing on cowpea produced a massive secondary root system and assembled its photosynthetic machinery for subsequent successful aboveground development, as reflected by the expression of amino sugar biosynthetic process, photosynthesis, and autophagosome assembly. In contrast, individuals growing on groundnut showed extensive shade-avoidance growth and -related gene expression, as well as poor organ development, suggesting parasitehost incompatibilities arising after attachment. This presentation will position the major transcriptional events, whether common or distinct in temporality and functionality, taking place during A. vogelii infection on compatible and non-compatible hosts. It will also discuss how A. vogelii uses host cells to form parts of its haustorium, as suggested by fine-grained transcriptomics and an analysis of thin sections and live scanning of host-parasite tissue interfaces. The data and basic knowledge of A. vogelii's host invasion and further development may help to improve management efforts for this weed in the future.

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#### Long-term associated Spiroplasma infection in neotropical Drosophila

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Microbial symbionts are shown to influence host phenotype, fitness, and behavior, but also the host controls its symbionts e.g., by domesticating and restricting them to specific organs. When this homeostasis between partners is interrupted by extrinsic and/or intrinsic factors, it gives a chance for symbionts and the host to find a new equilibrium that can lead to new phenotypes. Often, however, only the main prominent symbionts are studied but not so-called "hidden" ones that easily escape standard detection methods e.g., PCR. In such low-titer symbiosis the host is carrying the symbiont but their resulting phenotypes are context-dependent by host tissue tropism, low titer, and/or orthogenic stages. Spiroplasma and Wolbachia are both maternally inherited bacteria causing phenotypic consequences to their Drosophila host. Low-titer Wolbachia infections have been previously studied by our group in neotropical Drosophila by multicopy PCR marker or combinations with hyper-sensitive RNA-FISH immunohistochemistry. Similar to hidden Wolbachia infections, we also found Spiroplasma, another low-titer infection, in recently collected neotropical willistoni and saltans group Drosophila. Importantly this hidden Spiroplasma infection is usually hidden in adults and asymptomatic at room temperature but maintained in neotropical hosts. However, when flies were raised in elevated temperatures (>26°C) we could see a partial male-killing phenotype and infection status was possible to detect clearly in standard PCR. Interestingly based on the p18-marker this systemic Spiroplasma in neotropical Drosophila belongs to the S. poulsonii indicating long-term association of the symbiont in willistoni and saltans group flies.

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#### Life history predictions: sometimes intuitive, sometimes not

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Why should anyone fund theoretical evolutionary ecologists (like me)? I will argue that the reason is that intuition can lead us astray when making predictions about evolutionary processes. I will illustrate this with three different (theoretical) case studies: (1) The so-called life-dinner principle, where prey are supposedly ahead in any predator-prey arms race, because a prey individual will run for its life, while a predator only runs for its dinner. (2) The so-called Williams prediction, where selection works more strongly against senescence in species that live in relatively safe environments. (3) The coevolution of sex ratios and the frequency of parthenogenesis. Some of these confirm one's intuition more readily than others, though this of course depends on what one found intuitive in the first place. To make you curious: can you think of a trait that takes diagonally opposite values in birds versus stick insects?

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### Why does he care? Behavior-dependent conflict resolution during biparental care of a subsocial insect

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Parental care is a key driver of social evolution, providing a foundation for the consolidation of family life and advanced animal societies by conveying important benefits to offspring. The costs of care are often paid by the female but sometimes shared with a male during biparental care. Since parents' evolutionary interests differ, sexual conflict needs to be resolved for cooperation to occur. This conflict is well-studied in evolutionarily derived family systems like birds, but why biparental care initially emerged, and how the conflict is resolved when neither parent must remain with the young - a state which likely prevailed during the evolution of parental care - remains poorly understood. We investigated male investment into biparental post-hatching care in Nicrophorus vespilloides burying beetles which exhibit facultative biparental care on small vertebrate carrion with components such as offspring feeding and nest maintenance. Females exhibit most of these behaviors while males feed on and guard the carcass, but males also provide direct care to young. However, the mechanisms behind their varying investment into biparental care remain unknown. In our study, we investigated whether male care is repeatable between bouts (depending on personality) or depends more on the investment of the accompanying female. While some behaviors were driven by male personality, others depended on female investment, indicating a mix of negotiation, matching, and sealed-bid conflict resolutions. Overall, our findings show that different male care behaviors during biparental care likely derive from different selective pressures, highlighting that conflict resolution occurs repeatedly for different components of care.

# ResourceWars – Unraveling the entangled effects of predation and resource availability on the diversity and evolution of microbial communities

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Predation shapes ecosystems across macroscopic and microscopic scales, and often has a crucial impact on the diversity and evolution of prey communities. However, the interplay between predation and resource availability in shaping these communities remains poorly understood. Here, we tackle this issue by investigating how the facultative predator *Myxococcus xanthus* affects a defined prey community of 20 soil bacteria under varying resource conditions. Using three predator strains, we conducted a full-factorial, short-term evolution experiment by systematically varying predator presence, identity, and resource availability (low, medium, or heigh levels of eight defined carbon sources). Replicated predator-prey lines were transferred weekly to fresh medium over 10 weeks, and community diversity was monitored using metabarcoding. Our preliminary results reveal that predator presence and identity as well as resource availability all interact to determine community diversity. These findings underscore the importance of resource-mediated predator-prey dynamics in shaping microbial communities and provide insights into the mechanisms driving biological diversity in nature.

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#### Machine learning phylogenetics

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Understanding the relatedness of organisms is a fundamental question in evolutionary biology, often addressed through the construction of phylogenetic trees. Currently used methods for phylogenetic tree reconstruction face challenges with computational inefficiency due to everincreasing genomic datasets and complex evolutionary models to analyse the data. Machine learning approaches can tackle these limitations. In prior work, we demonstrated that neural networks trained on site pattern frequency distributions can predict quartet topologies with an accuracy comparable to the maximum likelihood method while being significantly faster (*Mayer et al 2024*). Here, we provide new evidence supporting the application of machine learning in phylogenetic tree reconstruction by comparing alternative machine learning methods such as gradient boosting, as well as faster and more accurate inferences through the use of dimensionality reduction techniques.

Kulikov N., Derakhshandeh F., Mayer C., 2024. Machine learning can be as good as maximum likelihood when reconstructing phylogenetic trees and determining the best evolutionary model on four taxon alignments. Molecular Phylogenetics and Evolution. https://doi.org/10.1016/j.ympev.2024.108181

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#### Supergenes as genomic substrate of divergent reproductive tactics

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Supergenes that arise from chromosomal inversion frequently provide the genomic substrate for divergent phenotypes, as inversions lock alleles of multiple genes together for joint inheritance and evolution. Although such supergenes only comprise a small proportion of the genome, they can increase intraspecific diversity substantially, especially when they harbour genes with major pleiotropic effects. In vertebrates, steroid-related genes provide strong candidates for such pleiotropic loci; as sex steroids are "phenotypic integrators" that impact multiple aspects of reproduction and life-histories. In ruffs, Calidris pugnax, an autosomal inversion polymorphism underlies three male mating morphs with striking differences in size, appearance, androgen levels, and social behaviours. The characterization of this inversion polymorphism has facilitated detailed studies into the proximate and ultimate mechanisms that help to maintain the exceptional genetic and behavioural diversity seen in these lekking sandpipers. The ruff supergene shows clear hallmarks of antagonistic pleiotropy; that is, the supergene variants do not only have profound fitness consequences for adult males but also for female reproductive success, development and survival of chicks. Knowledge about the supergene haplotype variation has also provided new insights into the molecular-physiological regulation of sex steroid levels in males. We found that observed differences in circulating androgens between male morphs are orchestrated by a single steroid-related gene, HSD17B2. This gene, which encodes 17-b-hydroxy-steroid dehydrogenase-2, shows signatures of positive selection in low testosterone morphs that resulted in isozymes with higher testosterone conversion rates than those present in the high testosterone morph. The morphs also show differences in expression in key tissues of androgen regulation matching their hormonal profiles. Taken together, our studies have identified different mechanisms of balancing selection and the accelerated evolution of few genetic loci being responsible for the maintenance of intraspecific diversity and the evolution of highly specialized reproductive tactics in this model species of sexual selection.

## SwarmGenomics: An Approachable and Comprehensive Pipeline for Whole-Genome Data Assembly and Genetic Analyses

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With the rapid advances in sequencing technologies, genomic data has become readily available, enabling researchers to explore evolutionary mysteries and assess the genomic health of populations. However, the complexity and computational demands of whole-genome sequencing (WGS) data analysis present significant obstacles - especially to those with little background in bioinformatics. SwarmGenomics addresses these challenges by providing a comprehensive, user-friendly pipeline designed for whole-genome data assembly and a variety of genetic analyses. The SwarmGenomics pipeline starts with the assembly of raw sequencing reads into a reference genome of a selected species. It then proceeds with seven modules, each providing valuable information on different aspects of the genomic data: heterozygosity analysis to measure genetic diversity within the population; runs of homozygosity (ROH) detection to identify regions indicative of inbreeding; Pairwise Sequentially Markovian Coalescent (PSMC) modelling to estimate historical population sizes and demographic history; analysis of unmapped reads to uncover potential novel sequences or contamination; repeat analysis to annotate and classify repetitive elements in the genome that may shed light on genomic degradation; mitochondrial genome assembly to reconstruct the mitochondrial DNA for insights into maternal lineage; and NUMT (nuclear mitochondrial DNA segment) analysis to identify nuclear integrations of mitochondrial DNA. We demonstrate these analyses on the giant panda (Ailuropoda melanoleuca) genome. By combining these analyses into a single, user-friendly pipeline, SwarmGenomics simplifies the process of obtaining comprehensive genetic information, making advanced genomic analyses accessible to researchers with varying levels of expertise. The modular design and straightforward workflow of SwarmGenomics also make it an great teaching tool, allowing students and new researchers to learn and apply genomic analysis techniques effectively.

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## Multiomics reveal associations between CpG methylation, histone modifications and transcription in a species that has lost DNMT3, the Colorado potato beetle

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Insects display exceptional phenotypic plasticity, which can be mediated by epigenetic modifications, including CpG methylation and histone modifications. In vertebrates, both are interlinked and CpG methylation is associated with gene repression. However, little is known about these regulatory systems in invertebrates, where CpG methylation is mainly restricted to gene bodies of transcriptionally active genes. A widely conserved mechanism involves the co-transcriptional deposition of H3K36 trimethylation and the targeted methylation of unmethylated CpGs by the de novo DNA methyltransferase DNMT3. However, DNMT3 has been lost multiple times in invertebrate lineages raising the question of how the links between CpG methylation, histone modifications and gene expression are affected by its loss. Here, we report the epigenetic landscape of Leptinotarsa decemlineata, a beetle species that has lost DNMT3 but retained CpG methylation. We combine RNA-seq, enzymatic methyl-seq and CUT&Tag to study CpG methylation and patterns of H3K36me3 and H3K27ac histone modifications on a genome-wide scale. Despite the loss of DNMT3, H3K36me3 mirrors CpG methylation patterns. Together, they give rise to signature profiles for expressed and nonexpressed genes. H3K27ac patterns, which show no association with CpG methylation, have a prominent peak at the transcription start site that is predictive of expressed genes. Our study provides new insights into the evolutionary flexibility of epigenetic modification systems that urge caution when generalizing across species.

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## Exploring the state-dependent Ornstein-Uhlenbeck model: a novel Bayesian joint inference approach to model continuous trait adaptation

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Adaptation is a prevailing hypothesis in continuous trait evolution at macroevolutionary scale, and is commonly modelled by an Ornstein-Uhlenbeck (OU) process. Under this framework, a continuous trait is adapting towards a species-specific optimal state, and the optima and strength of adaptation can vary across the phylogeny and over time along branches as discrete regimes. This framework can address two questions: (1) how continuous traits evolve, and (2) what drives continuous trait evolution. For example, how do fruit types drive climatic niche evolution in plants? How do dental morphology and digestive physiology of grazing and browsing mammals adapt to their diet differently? These questions can be answered using phylogenetic comparative methods. Traditionally, this is done in a stepwise approach – the discrete regime history is mapped onto a phylogenetic tree, followed by inference of OU parameters for the continuous trait evolution. A major limitation of this approach is that accounting for uncertainty in inferring regime history is computationally costly. To test the adaptation hypothesis in a more realistic way, we developed a flexible and computationally efficient state-dependent OU model in RevBayes, where the continuous trait and discrete regime history are inferred jointly using Bayesian inference. We simulated continuous traits under different state-dependent and state-independent OU processes to explore model performance for inferring state dependency. Results show that inference of OU parameters is relatively conservative, with a below-expected false positive rate. On the other hand, there is more power to infer state dependency of optima than there is for inferring phylogenetic halflives and stationary variances. Lastly, we demonstrate how diet preferences, in terms of food sources and diet selectivity, drive mammalian body size evolution using the state-dependent OU model. In conclusion, our novel Bayesian joint inference method can improve our understanding of continuous trait evolution by explicitly incorporating uncertainty of regime history inference.

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#### Investigating de novo gene emergence in six Drosophila species

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While it is well known that novel genes can emerge through the duplication of preexisting genes, it was discovered more recently that they can also emerge de novo from formerly noncoding genomic regions. So far, it is not completely understood how de novo genes can arise from scratch, nor is it clear how often they emerge, spread within a population and eventually gain a function. Still, the study of de novo genes remains a challenge due to the lack of datasets with consistent assembly and annotation pipelines as well as problems with the quality or completeness of the (in- and outgroup) data. Such problems can lead to incorrect estimations of de novo gene ages or numbers. We here aim to overcome these previous limitations by sequencing genomes and transcriptomes from 5-7 populations for each of in total six Drosophila species, with a divergence time of up to 40 million years and using a common assembly and annotation strategy. We then searched for newly expressed open reading frames (neORFs) in this dataset by making use of DESWOMAN, a newly developed software to detect de novo genes based on transcriptomes. This approach allows us to make use of a consistent dataset of very young as well as increasingly older neORFs, more likely to contain functional de novo genes. Further bioinformatic analysis will be carried out to investigate (i) the conservation of de novo genes across the phylogeny and (ii) the enabling mutations and other mechanisms that allowed these de novo ORFs to emerge.

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## Root system evolution and the role of auxiliary haustorial networks along the transition to plant parasitism

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Acquisition of a feeding organ that taps into the vasculature of another plant (haustorium) has been the essential event in the development of plant parasitism, and the need for a functional root system diminishes proportionally with the commitment to heterotrophy. Species in the Orobanchaceae family span the trophic spectrum of plant parasitism, and all build haustoria from root structures. Here, we demonstrate that obligate parasites like Striga hermonthica depend upon a network of subsequent attachments built from the adventitious root system to complete its lifecycle. We have used 3-dimensional X-ray scanning to show that the initial attachment often becomes non-functional in older individuals. We also found that mechanical damage to the host root supporting the initial connection resulted in increased transcription of glycolysis- and photosynthesis-related genes in the remaining attachments. However, we found only subtle transcriptional differences when comparing initial and subsequent haustoria of S. hermonthica, and the adventitious roots and the subsequent lateral haustoria in the obligate parasite Phelipanche ramosa. Using a refined master list of essential genes involved in root development, we have compared the expression of root genes in the genomic and transcriptional profiles of root tissue from eight species in the Orobanchaceae across the all major parasitic specialisations. Together, this study presents novel data regarding the patterns of haustorium and root system functions in parasitic plants, their expressional pathways across the trophic spectrum of parasitism and proposes a model of the role of root evolution during parasitic specialization.

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#### Blackcap migration - adaptation in time and space

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Understanding the genetics of bird migration is a long-standing goal in evolutionary biology. Blackcaps *Sylvia atricapilla* are ideal for this work as different populations exhibit enormous difference in migratory behaviour and little else. We characterize (i) phenotype, population structure and demographic history the blackcap, and (ii) identify sequence variants and signaling pathways that are associated with variation of the migratory phenotype. My talk will cover insight from classical studies on selection and cross-breeding experiments, tracking approaches in the wild, to finally introducing novel insight from using a de novo assembled genome of the blackcap as reference for large scale demographic study with different phenotypes across their breeding range.

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#### De-novo gene retention

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Genomes are dynamic and mutable much like the species they encode.. Through these genomic changes, de-novo genes, or non-genic regions of DNA which gain a function, can arise. De-novo genes are an important source of novelty for species to adapt and evolve. Many of these de-novo genes do not contribute to fitness initially and as a result experience a rapid birth-and-death life cycle. While there is a body of literature describing multiple mechanisms for how de-novo genes form, it remains unclear what mechanism(s) help them escape their rapid life cycle to persist and become retained within a genome. One potential mechanism we explore is if de-novo genes persist through indirect selection by hitchhiking and being in linkage with genes under positive selection. Using six Drosophila species with multiple populations per species as a model, we plan to test how often the de-novo genes are found near regions under selection. These results will indicate how prevalent this mechanism is for de-novo gene retention. If it is the primary way de-novo genes are retained, future research will focus on the *de-novo* genes located near positively selected regions. Alternatively, hitchhiking could represent only a minor portion of how these genes are retained. In this case, this will encourage future research to investigate alternative mechanisms to retain *de-novo* genes including direct selection on the *de-novo* genes themselves and genetic drift.

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#### Evolution in response to an abiotic stress shapes species coexistence

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Adaptation to abiotic stresses is pervasive and generally relies on traits that are not independent from those affecting species interactions. Still, how such evolution affects species coexistence remains elusive. We addressed this using populations of two herbivorous spider mite species (Tetranychus urticae and T. evansi) evolving separately on tomato plants hyperaccumulating cadmium, a stressful environment for the mites, or on control plants with nocadmium. Combinations of phenotypic analyses with structural stability theory predicted that adaptation of both species to cadmium allow them to coexist in that environment, whereas for cadmium-naïve mite populations the most likely outcome is competitive exclusion. The shift from exclusion to coexistence was due to an increase in structural niche differences caused by a simultaneous increase in intra and a decrease in interspecific competition. However, adaptation to cadmium did not affect species interactions and competitive outcomes in the cadmium-free environment, indicating that such evolutionary changes were environmentspecific. Outcomes of population dynamics with populations of the two species from each selection regime in each environment were fully compatible with model predictions. Therefore, evolution of single species in a new environment, even in absence of interspecific competitors, shapes species coexistence. Hence, population shifts to novel environments may have unforeseen evolutionary consequences for community composition and the maintenance of species diversity.

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#### The role of ZNF492 in neurodevelopment

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Although the human genome shares significant similarity with other primates, certain genetic features are unique to humans and contribute to the evolution of human traits. Species specific transcription factors are particularly interesting in elucidating evolutionary novelty, as they regulate the expression of numerous target genes. Krüppel associated box (KRAB) domain containing zinc finger genes (KRAB-ZNFs) are the largest family of transcription factors in vertebrates and are amongst the most rapidly evolving gene families. We identified ZNF492 as a human specific KRAB-ZNF gene, which presumably evolved via a segmental duplication of its now paralog ZNF98. ZNF492 is preferentially expressed in cortical neuronal progenitor cells (NPCs) of the foetal human cortex and could potentially play a role in regulating genes that confer human specific neurodevelopmental features such as an increase in the apical and basal progenitor cell populations. However, target genes and the precise functions of ZNF492 are not known yet. ZNF492 contains 13 zinc finger domains and differs from ZNF98 in 2 amino acid residues that potentially alter its binding site properties. Through this project, we aim to uncover the target genes of ZNF492 in neurodevelopment. As a starting point, we analysed publicly available CHIP-Exo data of human embryonic kidney cells expressing ZNF492. Our analysis revealed interesting targets with relevance to neuronal functions, such as SCN1A and SCN2B that encode voltage gated sodium channels required for the generation of action potential in neurons, or GRIK5 and DPP6 involved in synaptic transmission. To further reveal the binding sites of ZNF492 with a focus on neurodevelopment, we performed CUT&RUN sequencing using NPCs derived from human and chimpanzee induced pluripotent stem cells (iPSCs). Ultimately, our project intends to contribute to a deeper understanding of the genetic factors underlying human brain evolution by studying the role of human specific transcription factors.

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#### Automating High-Throughput Phenotyping of Daphnia: A Machine Learning Approach

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Phenotyping is crucial for understanding adaptation and fitness in response to environmental changes, yet traditional methods are labor-intensive and time-consuming. To address these challenges, we are developing a desktop application that leverages deep learning and computer vision to automate phenotyping in Daphnia, a keystone aquatic organism widely used in evolutionary and ecological studies. The application employs YOLO segmentation and convolutional models to detect key phenotypic features, such as the body shape, and measures their surface area. The body size is closely linked to Daphnia's fitness and adaptation to environmental pressures, such as predation or resource availability. The application will facilitate the study of phenotypic plasticity, trait adaptation, and fitness in response to environmental changes. Its modular design will support adaptation to other taxa, broadening its applicability to comparative studies, biodiversity assessments, and conservation efforts. By automating these measurements, the application enables high-throughput analysis, reducing manual effort and increasing the reproducibility of large-scale phenotypic studies. By streamlining phenotyping workflows, this tool will provide an efficient way to explore evolutionary patterns, enhancing our understanding of how organisms adapt to their environments.

### Timing and pattern of insect evolution - the 1kite tree 2.0

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Insects, as the most diverse group of animals on earth, are of profound evolutionary significance and play crucial ecological roles. Understanding their phylogenetic relationships and knowing their approximate divergence times provides insights into the evolution of key traits, ecological interactions and are interesting in their own right. Here, we present the largest insect phylogeny constructed to date from a transcriptomic data set, encompassing 1344 species. This phylogeny offers unparalleled resolution, revealing new insights into the evolutionary history of insects.

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## The molecular mechanism driving local adaptation to high-elevation habitats in East African honey bees (*Apis mellifera*)

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A major objective in population biology is to understand the evolutionary processes that underpin differentiation within species. One pivotal mechanism is local adaptation, whereby organisms evolve traits that enhance their survival and reproductive success in particular environments. The honey bee (Apis mellifera) in East Africa provides an illustrative example of this phenomenon, with highland populations exhibiting distinct phenotypes, including pigmentations and behavioural traits, in comparison to their lowland counterparts. Despite the above described phenotypic differences, the genetic differentiation between the highland and lowland bees is low, with the exception of two chromosomal regions on chromosome 7 (r7) and chromosome 9 (r9), previously identified as chromosomal inversions. The rarity of these inversions in lowland bees suggests that they play a key role in the adaptation to high elevations. In the present study, we analysed whole-genome sequences from honey bees in Western Uganda and Kenyan populations of different elevation habitats. Our findings corroborate the hypothesis that chromosomal inversions r7 and r9 are linked to highland adaptation in both regions, though with notable regional variations. Additionally, genome-wide selection scans identified further genomic regions that may be involved in high-altitude adaptation. Transcriptome data from highland and lowland bees in Uganda yielded new insights into the differential expression of genes linked to behavioural traits and oxygen consumption. These findings elucidate the interplay between r7, r9, and gene expression, uncovering a complex regulatory network that underlies A. mellifera adaptation to highelevation environments. Ongoing functional analyses of candidate genes will enhance our understanding of transcriptional elements that drive adaptation, elucidating the interaction of regulatory pathways in evolutionary processes of phenotypic differentiation in honey bee populations.

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### Sex & Germs & Speciation: The Wonderful World of Neotropical Wolbachia

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Selfish genetic elements (SGEs) like transposons, viruses, bacteria, archaea and protists are universal life entities with the capacity to replicate faster than the host and to coevolve tightly by fluctuating waves of conflict and cooperation. In the light of the Holobiome concept, the phenotype of an organism is formed not only by its nuclear and organelle genomic compounds but also by the genomic entities of its cohabitating SGE symbionts. Thereby, SGEs are now considered as important factors to drive along the genetic diversity and speciation of their hosts, even within short evolutionary periods of time. As shown by the latitudinal diversity gradient, tropical organisms have a much higher diversification and speciation rate than temperate ones, and thereby are ideal systems for studying the tempo and mode of SGEdriven host speciation under experimental conditions. Drosophila paulistorum spp. is a neotropical species complex that became famous since the 1960s by Dobzhansky and Ehrman as a reference model system to study the causes and consequences of incipient speciation in nature, but also under lab-controlled conditions. We show that the maternally-transmitted endosymbiotic Wolbachia bacteria are fixed mutualistic entity of all D. paulistorum flies, which are restricted to defined functional host tissues by autophagy, and importantly, direct sexual mating behavior of both sexes and hence drive reproductive isolation between closely related neotropical fly species.

# Indirect genetic effects increase the heritable variation available to selection and are largest for behaviours: a meta-analysis

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The evolutionary potential of traits is governed by the amount of heritable variation available to selection. While this is typically quantified based on genetic variation in a focal individual for its own traits (direct genetic effects, DGEs), when social interactions occur, genetic variation in interacting partners can influence a focal individual's traits (indirect genetic effects, IGEs). Theory and studies on domesticated species have suggested IGEs can greatly impact evolutionary trajectories, but whether this is true more broadly remains unclear. Here we perform a systematic review and meta-analysis to quantify the amount of trait variance explained by IGEs and the contribution of IGEs to predictions of adaptive potential. We identified 180 effect sizes from 47 studies across 21 species and found that, on average, IGEs account for a small but statistically significant amount of phenotypic variation (0.03). As IGEs affect the trait values of each interacting group member and due to a typically positive although statistically nonsignificant – correlation with DGEs (r<sub>DGE-IGE</sub> = 0.26), IGEs ultimately increase trait heritability substantially from 0.27 (narrow-sense heritability) to 0.45 (total heritable variance). This 66% average increase in heritability suggests IGEs can increase the amount of genetic variation available to selection. Furthermore, whilst showing considerable variation across studies, IGEs were most prominent for behaviours, and to a lesser extent for reproduction and survival, in contrast to morphological, metabolic, physiological, and development traits. Our meta-analysis therefore shows that IGEs tend to enhance the evolutionary potential of traits, especially for those tightly related to interactions with other individuals such as behaviour and reproduction.

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## Larvae in the fast lane: faster growth, not higher mortality, in fungus-exposed offspring of a family living beetle

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Animal taxa exhibiting post-hatching care can be found throughout the animal kingdom. During this period, parents aggregate with their offspring and allow them to invest their resources into growth and development as parents take over energy consuming tasks. For instance, studies show that food provisioning and social immunity by parents can alleviate the costs of an offspring's immune response to pathogen exposure. However, this issue has rarely been explored from the offspring' side, especially in species where offspring shows plasticity regarding dependency on parental care. Here, we raise the question of how offspring is affected by pathogen exposure if they have access to social immunity through a caring parent. Parents of *Nicrophorus vespilloides*, a species exhibiting facultative post-hatching care, control the carcass microbiome by coating it with their antimicrobial exudates, stopping further decay and protecting their offspring from potential pathogens. In this study, we exposed N. vespilloides offspring to a generalist entomopathogenic fungus, Beauveria bassiana, while manipulating the parental care they received. We monitored offspring performance parameters throughout their development and found, curiously, that regardless of parental care, larvae showed an in-crease in mean weight and growth rate after being exposed to the pathogen, while their survival and long-term immunity were unaffected. Simultaneously, our results indicate that females in-vest fewer resources into their offspring if they have been exposed to the pathogen. Overall, we show that offspring of facultative subsocial species do not respond differently to pathogen exposure regardless of their parents' aid.

# Phenotypic and early transcriptome differences across different populations and their hybrids in *Solanum chilense*.

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Drought stress presents a significant challenge to agriculture globally due to its limiting effects on crop yields, accompanied by climate change. Wild tomatoes (Solanum section Lycopersicon) are a good model system for studying plant demography and local adaptation to abiotic stresses. Demography and adaptation are important interacting factors that determine the evolution of plant species. Adaptation to the local environment is a primary force driving morphological evolution and allopatric speciation. Solanum chilense is considered one of the most promising genetic resource for tomato selection to biotic and abiotic stresses. In this study, we compared the effect of drought under controlled climatic conditions in different populations of Solanum chilense, each characterized by distinct local habitat. Seven accession of parental populations LA4107, LA3111, LA1963, LA4330, LA4117A, LA2932, LA2755 from different local habitats i.e. coast, central, and highland were used during the experiment. We measure the germination rate of seeds, plant growth parameters, physiological responses, and morphological characters. Our result demonstrated strong variability among and between accessions, indicating the polygenic and multiple trait nature of the tolerance of Solanum chilense to water stress and adaptation to local habitat. Furthermore, to investigate the divergence and the genetic basis of reproductive isolation between the most southern populations (coastal and highland) of S. chilense on either side of the Atacama desert, we performed crosses between these parental populations. We successfully produced both F1 and F2 generations and indeed observed strong compatibility between the crosses. We notably observed a pronounced maternal effect in the crosses between coastal and highland populations LA4107× LA4117A. To further validate and decipher the genetic basis of this maternal incompatibility effect, we are conducting an RNA-Seq experiment focusing on early root and stem developmental stages. We aim to find out differentially expressed genes responsible for developmental differences between the reciprocal crosses.

# The Crazy X: Genetic architecture, variation, and evolution of the X-chromosomal SpermiR clusters in Mus musculus subspecies

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Meiotic recombination ensures proper segregation of homologous chromosomes, ensuring fertility and avoiding aneuploidy. It also introduces genetic variation, characteristic to sexual reproduction. The recombination rate varies across species, individuals, sexes, chromosomes, and even regions within a chromosome. In mice and humans, PRDM9 determines the nonrandom recombination sites and is the only known hybrid sterility gene. However, hybrid sterility is an oligogenic trait. The extended hybrid sterility locus on the mouse X-chromosome (HstX2) houses a family of microRNAs called the SpermiRs, which are implicated in male sterility and are also candidate recombination regulators. We investigate the evolution of the genetic and epigenetic components in the HstX2 locus that controls hybrid male sterility and meiotic recombination rate and the evolution of the interconnection and modulation of these phenomena from the same locus. For the first time, we resolved the genetic architecture of HstX2, uncovering highly repetitive gene regions from genome assemblies of varying qualities using custom secondary mappings, in 16 strains of mice, spanning 3 Mus musculus subspecies. We identified novel microRNA genes and studied their secondary structures, copy number variation, and targets. We characterized the intra-subspecific and within-strain variation, the birth, death, accelerated expansion, and divergence of natural alleles of epigenetic meiotic recombination regulators in mice, and the evolution of the HstX2 locus. We provide a comprehensive evolutionary description of the nature and dynamics of the epigenetic meiotic recombination regulators in terms of diversity, hybrid sterility, and speciation through post-zygotic barriers, with implications in male fertility and contraceptives. Our research preludes that the evolution of the microRNA clusters by copy number variation controls hybrid male sterility and meiotic recombination rate in a dosage-dependent manner and that single nucleotide polymorphism allows control of different traits by binding to different targets in the different intrasubspecific hybrids. We now extend the investigation to humans.

#### Comparing great apes reveals human-specific ZEB2 roles in neural development

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Despite nearly identical protein-coding sequences, humans differ from other primates in a number of traits. Understanding their evolution requires investigating changes in transcriptional regulation. However, comparative functional studies of transcription factors (TFs) across primates are still rare. Here we focus on ZEB2, a ubiquitously expressed TF critical for immune system and neural development, to understand its regulatory divergence across great apes. We show that, in addition to conserved ZEB2 targets, human ZEB2 is distinct in regulating a larger repertoire of genes implicated in neuronal development. In particular, using ChIP-Seq in B-lymphoblastoid cells from humans, chimpanzees, and orangutans, we found ZEB2 binding sites predominantly near transcription start sites in all species, albeit binding sites for genes with neuronal functions have higher affinity for human ZEB2. ZEB2 knockdown in the same cells followed by transcriptome profiling discovered human-specific regulatory differences, especially in genes linked to nervous system development. Further investigation using singlecell RNA-Seg and RNA-Seg data from developing brain organoids revealed cell-type-specific differences in ZEB2 expression and regulated genes between humans and other apes, most pronounced in ventral progenitors and neurons. Moreover, human-specific targets are enriched for non-coding genes, suggesting expansions in ZEB2's regulatory network. Our findings provide new insights into the functional divergence of a TF across closely related species, highlighting the role of ZEB2 in the evolution of the human brain. This work underscores the importance of regulatory shifts in shaping human-specific traits and offers a deeper understanding of the impact of transcriptional changes on phenotypic evolution.

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# Long-term maintenance of transposable element activity is achieved through regulation by nonautonomous elements

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Transposable elements (TEs) are mobile genetic elements that can invade and persist in host genomes. They are broadly classified into two types: autonomous TEs, which encode the enzymes required for their mobility, and nonautonomous TEs, which depend on the transposition machinery of autonomous TEs for their transposition. Nonautonomous elements have been proposed to regulate the abundance of transposable elements, which may account for the sustained transposition activity over evolutionary timescales. However, earlier modelling studies reveal that inter- actions between autonomous and nonautonomous elements often lead to the loss of one type. In this study, we analyze a stochastic model that enables the stable coexistence of autonomous and nonautonomous retrotransposons. We determine the conditions required for this coexistence and derive an analytical expression for the stationary distribution of their copy numbers, showing that nonautonomous elements regulate stochastic fluctuations and regulate the number of autonomous elements in stationarity. We find that the stationary variances of each element can be expressed in terms of the average copy numbers and their covariance, facilitating data comparison and model validation. These results indicate that the sustained transposition activity of transposable elements, regulated by nonautonomous elements, is a possible evolutionary outcome. This could, for instance, explain the long co-evolutionary history of autonomous LINE1 and nonautonomous Alu element transposition in human ancestry.

#### Uncovering Introgression Events in Potato Domestication via TE Landscape Analysis

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The common potato (Solanum tuberosum L.), a globally important crop, is a tetraploid species originating from a diverse group of over a hundred wild potato species native to South America. Domesticated around 10,000 years ago in the southern Andes of Peru, potatoes were later introduced to Europe in the 16th century, from where they spread worldwide. This complex evolutionary history has been shaped by processes such as introgression from wild species into cultivated varieties, mediated through numerous hybridization and polyploidization. These events have driven genome evolution in potatoes and may have influenced bursts of transposable element (TE) activity—mobile genetic elements that proliferate within genomes and serve as markers for historical genome-merging events. In this study, we leverage an extensive dataset of 815 samples, comprising 154 cultivars and 78 wild species, along with de novo phased assemblies from 12 cultivated potatoes, to investigate TE landscapes as indicators of introgression events in cultivated potatoes. Our analysis focuses on the presence and distribution of distinct TE populations from various wild species, with emphasis on comparing TE abundance in introgressed genomic regions. By assessing TE abundance and examining genetic diversity within TEs, we aim to infer the relative timing of introgression events. This approach offers valuable insights into the genomic consequences of introgression during domestication, particularly in relation to the population dynamics of TEs and their role in shaping the adaptive potential of cultivated potatoes.

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## Cryptic diversity in Eurasian minnows: whole-genomes reveal population structure and resolve incomplete lineage sorting in the mitochondrial *COI*-marker

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Recent studies have revealed previously unknown cryptic diversity within Eurasian minnows (Leuciscidae – *Phoxinus* phoxinus), challenging its monotypic classification in Europe (Palandačić et al., 2015, 2017). These findings have profound implications for conservation and taxonomy, particularly in countries like Germany, where multiple *Phoxinus* species coexist. The recognition of multiple genetically distinct lineages within *Phoxinus* led to a revision of German freshwater conservation laws to reflect the conservation needs of each recognised species (Freyhof et al., 2023).

The existence of several species in Germany and the historical implementation of stocking and reintroduction programmes prompted us to understand the distribution and population structure of the different *Phoxinus* species in Germany and neighbouring countries. We conducted population structure analyses using our annotated, chromosome-resolved reference genome (Oriowo et al. 2024) and whole-genome datasets of more than 6 million SNPs from over 300 *Phoxinus* individuals from various catchments in and around Germany as well as base don barcodes retrieved from own samples and NCBI.

Instead of the four recognized *Phoxinus* species (*P. phoxinus*, *P. csikii*, *P. morella*, and *P. marsilii* [not included in this study]) at least 5 genetically distinct *Phoxinus* lineages are present in Germany plus an additionally new lineage identified in France: populations of *P. phoxinus* (clade 10) from Seine and Meuse/Lower Rhine and *P. csikii* (clade 5b) from Danube and Lower Rhine are genetically clearly distinct based on whole-genomes and form their own populations. The Middle-Lower Rhine boundary seems to be distribution boundary for *P. phoxinus* 10 "Meuse-type" and *P. csikii* 5b "Rhine-type".

This demonstrates that COI-barcodes are not sufficient for *Phoxinus* spp. delineation. Whole genome re-sequencing has proven to be useful for biodiversity assessment and offers the possibility to decipher evolutionary significant units.

# The role of individual variation in herbivore foraging behaviour for the maintenance of polymorphism in plant defences

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Many plant species exhibit polymorphism in their defensive traits against herbivores even on a local spatial scale. One potential mechanism for maintenance of such polymorphism is negative frequency-dependent selection mediated by adaptive foraging of herbivores. Using individual-based simulations, we investigate the role of individual variation in herbivore foraging behavior on population dynamics and the maintenance of polymorphism in plants. As a model, we use plant-herbivore interactions between Arabidopsis halleri subsp. gemmifera and the leaf beetle Phaedon brassicae. Several laboratory and semi-field experiments conducted earlier for this study system suggest that conspecific neighbour effects triggered by adaptive foraging of herbivores can explain defensive dimorphism in A. halleri. We investigate how these dynamics are affected by different patterns of herbivore behaviour variation as well as by sudden changes in these patterns that could occur as a consequence of the environmental change.

#### Can a natural Daphnia magna population (rapidly) adapt to a new predator?

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Predation is a key factor shaping ecosystems, and the invasion of a fish predator can severely impact freshwater habitats by altering zooplankton communities. Daphnia are famous for showing several defence strategies when facing fish, including morphological, life-history and behavioral changes. The selection pressure by predation on Daphnia populations is typically strong and phenotypic changes in response to changes in predator regime rapid and often complex. The underlying genetic architecture has been described as polygenic and several hundreds of single nucleotide polymorphisms (SNPs) are involved in adaptation to fish predation. Here, we study a recent fish invasion to a previously fish-free gravel pit lake in Northern Germany and its consequences for the local Daphnia magna population. After its establishment in 2006, the lake harbored a rich zooplankton community including Daphnia magna and Daphnia pulex. In 2019, first individuals of the fish species common sunbleak (Leucaspius delineatus) were detected and the fish population increased massively over the following years. We analyzed the consequences for the zooplankton community in general and, more specifically, the genomic and phenotypic characteristics and changes in the local *D. magna* population. We further compare our results to predictions made from population genetic simulations mimicking the scenario of fish invasion as well as to data from other European D. magna populations experiencing selective pressure by fish predation.

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#### The impact of parasite diversity on the evolution of host immunity

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Many modern human diseases result from maladaptive responses to new environments. The recent reduction of parasite diversity in modern human populations for example is suspected to be a primary cause for the increase of autoimmune disorders. However, the long-term evolutionary consequences of decreased parasite diversity on the host immune system are not well understood. We used the cavefish Astyanax mexicanus to understand how loss of parasite biodiversity influences the evolutionary trajectory of the vertebrate host immune system by comparing river with cave ecotypes. Using an image-based immune cell phenotyping approach and single-cell RNA sequencing, we identified a shift in the overall immune cell composition in cavefish as the underlying cellular mechanism associated with altered immune responses. The shift results in an overall decrease of innate immune cells mediating inflammation and cellular immune responses such as phagocytosis (i.e., neutrophils and monocytes). Furthermore, using a QTL approach, we were able to identify the genetic loci that drives the differences in immune cell composition. Together with the scRNAseq data we were able to identify genes that potentially control the decreased production of innate immune cells in the hematopoietic tissue of cavefish. These findings reveal that A. mexicanus is wellsuited model to study the evolution of immunity in response to environmental changes.

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## Longterm genomic consequences of thelytoky in the Cape Honeybee *Apis mellifera* capensis

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The Cape Honeybee Apis mellifera capensis is a subspecies of the Western Honeybee, native to South Africa. Many Cape Honeybee workers are able to produce female offspring from unfertilized eggs via thelytoky with central fusion, a form of parthenogenesis where diploidy is restored by the fusion of meiotic products. While this mode of reproduction may be adaptive, e.g. to re-queen the colony from worker-laid eggs, it is predicted to result in a loss of heterozygosity (LOH) and therefore in a reduction of genetic variation and fitness. Despite this detrimental effect, a permanently thelytokous, parasitic lineage of Cape bees is persisting for over 30 years, while large fractions of its genome retained heterozygosity. Yet it remains unclear whether this is a consequence of reduced recombination or strong selection against homozygotes and what long-term effects there are. Using whole genome sequence data of families as well as developmental and population time-lines of South African Honeybees from the native population and the parasitic lineage, we estimate recombination rates in queens and workers, the level of selection against LOH during development and the long-term consequences of thelytokous reproduction in the parasitic lineage. The Cape Honeybee represents an ideal study system to investigate mechanisms of preserving heterozygosity despite asexual reproduction and serves as a model for other thelytokous organisms.

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## Variation in temporal transcription factor expression underlies differences in eye size in *Drosophila simulans* and *D. mauritiana*

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Compound eye size in insects, determined by ommatidia number and size, is a key visual adaptation to diverse environments and lifestyles. Despite its ecological importance, the genetic and developmental mechanisms controlling eye size evolution remain poorly understood. We identified an X-linked region contributing to larger eyes in *Drosophila mauritiana* compared to *D. simulans*, primarily due to increased ommatidia size. We combine fine-scale mapping and gene expression analysis to investigate candidate genes within this region. We report earlier expression of the developmental gene *orthodenticle* (*otd*) during ommatidial maturation in *D. mauritiana*. Functional analyses in *D. melanogaster* confirm its role in ommatidia size and organization. Furthermore, a species-specific *otd* eye enhancer recapitulates the observed heterochronic expression shift. Finally, we identify potential downstream targets of Otd differentially expressed between *D. mauritiana* and *D. simulans*. Our findings implicate altered *otd* expression timing in the evolution of compound eye size, providing novel insights into the genetic regulation of visual system adaptation in insects.

#### Carbon Utilization and Resource Allocation: Drivers of Metabolic Cooperation

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Microorganisms exist in complex communities and rely on the exchange of essential metabolites to sustain growth of the community. Such interactions determine the structure, function and evolution of a given community. While metabolic cross-feeding interactions have been shown to provide evolutionary and ecological advantages, the underlying biochemical and regulatory mechanisms remain poorly understood. Several studies highlight the importance of metabolic trade-offs in shaping the adaptive evolution cross-feeding interactions. This study investigates the impact of trade-offs resulting from the underlying metabolic architecture of the bacteria on the assembly and evolution of microbial communities and whether these tradeoffs can be broken by crossfeeding. Coexisting bacterial species, can have different resource preferences, which can affect the biosynthetic costs for producing metabolites by affecting the distribution of fluxes. In the first phase of this work, we characterized the carbon utilization profiles of these strains across diverse carbon source conditions. Building on these findings, we are now quantifying amino acid production and consumption under varying carbon sources to elucidate metabolic constraints and resource allocation strategies. Pairwise and community-level coculture experiments will be performed to determine that carbon source associated - tradeoffs influence cross feeding interactions and strain fitness. This work will provide fundamental insights into the biochemical and metabolic factors driving division of labor in microbial communities. Such a quantitative understanding of the rules governing community assembly and function offers a framework for designing microbial consortia for medical or biotechnological applications.

## **Evolution of Wolbachia nutritional symbiosis –The Enigmatic Case of the Biotin Operon**

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The obligate intracellular endosymbiont Wolbachia (Alphaproteobacteria) is considered the most abundant symbiont with an estimated 40 % of Arthropod species infected. In most Arthropods, Wolbachia exerts negative fitness effects on its host, causing reproductive aberrations which facilitate the symbionts transmission. In others, it was demonstrated that Wolbachia acts as a nutritional mutualist. For Insects like the bedbug C. lectularius or other Hemipterans, the monotonous and nutrient depleted diet explains the need for a nutritional symbiont, which could be demonstrated in rearing experiments. A recent genomic analysis of over 200 Wolbachia genomes however revealed the presence of an operon responsible for biotin (vitamin B7) production to be present in 14 Wolbachia of different insect hosts. Strikingly, eight of these Wolbachia were symbionts of solitary bees. Despite the fact that solitary bees are a diverse and ecologically important group of Insects, comprising 604 different species in Germany alone, the role and evolution of biotin supplementation by Wolbachia in bees remains enigmatic. The aim of my project is to investigate the distribution, evolutionary history and functionality of this biotin operon in order to understand the evolution of a potential nutritional mutualism and elucidate the relationship between Wolbachia and their solitary bee hosts. For this, a comprehensive screen for Wolbachia symbionts carrying the biotin operon was performed. This revealed the phenomenon to be even more widespread than previously thought. A large set of Illumina-Sequencing data for the biotin operon and surrounding gene regions of Wolbachia from a variety of bee hosts will furthermore be subject to phylogenetic analysis of bees and their biotin producing symbionts while phage derived mobile genetic elements on the Wolbachia genome will be investigated as potential vectors for the spread of the operon.

### How to offset aging by growing longer

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Within the cells of aging organisms often lie damaged proteins, with misfolded structures that cluster together as aggregates. Not only is this a hallmark of mammalian aging, correlating with the onset of age-related diseases, as it might represent a conserved aging marker across cellular organisms. Bacteria are no exception. In the rod-shaped Escherichia coli, aggregates of misfolded proteins accumulate in the old poles of mother cells over generations. However, recent evidence has questioned whether these aggregates correlate with a progressive loss of fitness, suggesting they could instead have a neutral or even protective role. We addressed this conflict through single-cell microscopy and microfluidics, quantifying the formation and inheritance of protein aggregates over generations of mother and daughter cells. We found that the main factor driving a fitness decline is not the presence of an aggregate, but how much of the intracellular space it occupies. The accumulation of such damage corresponds to a decline in gene expression and the presence of fewer newly-synthesized proteins in maternal old poles, producing the aging phenotype. Surprisingly, we found a compensatory mechanism where the mother cell preserves its physiologically active area by growing longer and dividing with morphological asymmetry — indicating that the divisional asymmetry of E. coli goes beyond physiological effects. These results propose a synthesis of aging mechanisms operating in bacterial lineages, where the accumulation of intracellular damage produces a deterministic asymmetry of growth rates, gene expression, and inheritance of freely-diffusing proteins. More importantly, by showing that protein aggregation is a deterministic driver of bacterial aging and a source of phenotypic asymmetry, our findings strengthen the idea that this process represents a hallmark of aging across cellular organisms.

## How beetles tick- Insights into the circadian system of the model species *Tribolium* castaneum

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Circadian clocks regulate daily rhythms and enhance the fitness of organisms by allowing them to anticipate, rather than simply react to, 24-hour changes in their environment. These clocks rely on transcriptional-translational feedback loops, that are evolutionarily highly conserved. However, the molecular components and their behavioural outputs often vary between species. Although beetles are the most species-rich animal taxon, their circadian systems remain poorly understood. Here, we present a comprehensive analysis of circadian activity patterns and first insights into their molecular underpinnings in the model beetle species *Tribolium castaneum*.

Unlike most species typically used in chronobiological studies, this beetle shows considerable inter-individual variation in the rhythmicity of its locomotor activity patterns. While some individuals showed clear behavioural rhythms, a noteworthy proportion showed arrhythmic activity. Further, we aimed to compare the molecular basis of the observed behavioural rhythms with the situation in other species. Therefore, we used RNA interference in *T.castaneum* to target core clock genes, that have different functions among insects. Knockdowns of *Period* and *Cryptochrome 2*, but not of *Timeless*, disrupted behavioural rhythms. This indicates a clock which most closely resembles the one described for Heteropterans, with major differences from the clocks described for other holometabolous insects as *Drosophila melanogaster*.

Our results highlight the extent of individual variation in clock-driven behaviour in *T. castaneum*, improve our understanding of beetle chronobiology, and underline the evolutionary flexibility of insect clocks.

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### How antisense RNAs can evolve to encode novel proteins

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Protein coding features can emerge *de novo* in non-coding transcripts, resulting in emergence of new protein coding genes. Studies across many species show that a large fraction of evolutionarily novel non-coding RNAs have an antisense overlap with protein coding genes. The open reading frames (ORFs) in these antisense RNAs could also overlap with existing ORFs. In this study, we investigate how the evolution an ORF could be constrained by its overlap with an existing ORF in three different reading frames. Using a combination of mathematical modeling and genome/transcriptome data analysis in two different model organisms, we show that antisense overlap can increase the likelihood of ORF emergence and reduce the likelihood of ORF loss, especially in one of the three reading frames. In addition to rationalising the repeatedly reported prevalence of *de novo* emerged genes in antisense transcripts, our work also provides a generic modeling and an analytical framework that can be used to understand evolution of antisense genes.

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#### The influence of transposable elements on genome architecture and evolution in ants

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Transposable elements (TEs) are major players in evolution and have substantially shaped the structure and function of all genomes, ranging from bacteria to humans. Additionally, TE-genome interactions can be highly dynamic and lead to large-scale genome rearrangements, even between closely related organisms. In the ant genus Cardiocondyla, genome evolution has led to an extraordinary TE distribution, separating slowly evolving TE-poor from highly distinct fast evolving TE-rich regions. By comparative genomic studies across several closely related species of Cardiocondyla, we unravel how such extreme genome architecture can evolve, how it affects genome evolutionary dynamics, and how it affects species diversification at the genomic and other levels. Our analyses reveal spectacular degrees of genome rearrangements within the genus, resulting in highly distinct genome structures between species. We conclude that the unusual TE distribution in Cardiocondyla likely both contributes to and results from these extraordinarily dynamic genome evolutionary changes.

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### Evolutionary footprints of parental care in weevil genomes

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Sociality in animals can be described as a continuum from subsociality, comprising parental care, to eusociality, comprising cooperative brood care, overlapping generations and division of reproductive labour. Research into the molecular evolution of sociality has so far focused on eusociality in Hymenoptera and termites. We, however, are interested in the evolution of lower social complexity, namely parental care, as a potential first step in the evolution of more complex social phenotypes. There is a longstanding hypothesis that social behaviour affects genetic variation. Parental care for example can buffer the effects of mildly deleterious mutations and thus shelter the offspring from selection. Furthermore, the evolution of subsociality is expected to lead to changes in gene expression regulation rather than genomic changes, however, this remains to be tested. Here, we investigate genomic footprints of parental care in weevils (Curculionidae, Coleoptera). Within weevils, parental care evolved at least twice, and one species is classified as eusocial, making this beetle family a great study system for the evolution of (sub)sociality. We are applying genomics approaches to investigate changes in gene family size and selection regimes across the species tree. We observe convergent changes in the size of gene families related to transcription, as well as a relaxation of selection on genes related to gene regulation in the branches where parental care evolved. Our observations across the two origins of parental care in weevils support the hypothesis that changes in regulation of gene expression correlate with the evolution of parental care. In the future, we will broaden this study by adding newly assembled and annotated genomes of weevils of different social complexity, including eusociality, to the dataset.

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#### Disentangling the sex effects on shared copulatory traits in Gryllodes sigillatus

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Shared sexual or interaction traits refer to any aspect of sexual behaviour that involves a direct interaction between male and female mating partners, and thus all traits leading to sexual conflict. In order to understand the evolution of traits under sexual conflict, it is important to consider the contribution of both sexes to these traits. In most species, these traits are primarily studied through a male perspective, thereby ignoring the contribution of the female partner. On the other hand, while intuitively, the value of shared traits should be influenced by both sexes, this need not be true if one sex has evolved predominant control. Aside from mating partners, individuals are also influenced by their social environment (eg. density of conspecifics around). To bring all these aspects into account, we wanted to test how rearing density treatments given to both males and females, influenced the variation in copulatory traits such as copulation duration and sperm transfer in *Gryllodes sigillatus*. We found a highly significant effect of male treatment on the number of sperm transferred (confirming that males have a higher control over this trait as we had hypothesized) but no such effect of either sex on copulation duration. Interestingly, we also did not find a significant correlation between the two traits as is expected in most species. These results, highlight the complex nature of copulatory interaction traits and how it is important to study the contribution of both sexes along with their social environment to understand the variance of such behaviours in a population.

#### Causes and Mechanisms of Sex Ratio Distortion in Altica lythri Flea Beetles

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Wolbachia infections are well known to cause sex ratio distortions, but these may also arise from intergenomic conflicts. In Altica lythri, a flea beetle species, three divergent mitochondrial haplotypes (HT1, HT2, HT3) have been identified, two of which are associated with normal sexual reproduction. However, females of the HT1 haplotype exclusively produce all-female offspring, despite requiring copulation with males of other haplotypes for successful reproduction. The mechanisms underlying these reproductive anomalies are the focus of our ongoing research project. Paternity studies confirm a gynogenetic reproduction mode of HT1 females, where males contribute no genetic material to the offspring. Genetic analyses with Xlinked markers in quantitative real-time PCR further revealed that some HT1 females possess only a single X chromosome instead of an XX karyotype, suggesting feminized males in this haplotype. Whether Wolbachia plays a role in this reproductive anomaly — either through active infections or through nuclear integration of Wolbachia genes — is currently being investigated by antibiotic treatments of females and larvae. Alternatively, conflicts between mitochondrial and nuclear genomes may contribute to the skewed sex ratio. To investigate this, PacBio sequencing was performed for all haplotypes, enabling the assembly of distinct Wolbachia strains and subsequent comparative analyses. Our study highlights the fascinating interplay between symbionts, host genomes, and reproductive strategies in A. lythri. Deciphering these processes contributes to our understanding of the evolution of sex determination systems and intergenomic conflicts.

### **Cavefish Challenge the Limits of Starvation Tolerance in Vertebrates**

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Starvation and malnutrition persist as leading causes of mortality globally, accounting for over nine million deaths annually, disproportionately affecting children. In response to food scarcity, animals in the wild have evolved a spectrum of adaptive survival strategies. These include hibernation or torpor to extend life for extended periods without food, and hyperphagia, which allows them to survive long intervals between feedings. However, the general time for an active animal's survival without food is usually less than one year. Our study challenges this assumption by presenting evidence that cavefish can actively survive without food for over sixteen months. This finding is particularly remarkable given that closely related surfacedwelling fish can only survive a maximum of five months of starvation, even with prior unrestricted access to food. This increased starvation tolerance is evident from the larval stage in cavefish, suggesting a cellular mechanism of adaptation rather than solely a behavioral one. To probe the cellular basis of this extended starvation resistance, we developed liver-derived cell lines from both cavefish and surface fish populations. These cell lines were subjected to starvation by culturing in serum-deprived conditions. Our results show that cavefish cells could endure these harsh conditions up to fivefold longer than their surface counterparts. This suggests an innate cellular resilience in cavefish against starvation, potentially offering new insights into vertebrate starvation resistance mechanisms. Further, our RNA-seq analysis under starvation conditions has identified significant signaling pathways implicated in this survival strategy. With the establishment of an in vitro system, we are now poised to functionally dissect the roles of these pathways in starvation resistance.

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#### Adaptive radiation and social evolution of the ants

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Ants are among the world's most successful lineages of animals. Over the last 150 MY of evolution, they became dominant parts of terrestrial ecosystems and diversified into over 15,000 extant species in 340 genera. In 2017, we initiated the *Global Ant Genomics Alliance* (GAGA) to generate large comparative genomic, transcriptomic, phenotypic and life history data to address fundamental questions about the evolution of the ants. The analyses of more than 130 high-quality genomes that I will present here have allowed unprecedented insights into the genetic underpinning of ant social traits, their adaptive radiation, and their genome evolutionary history.

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# Understanding the Ricefish Plug: Architectural Insights through Spatial Transcriptomics

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The Stress Induced Evolutionary Innovation (SIEI) model suggests that ancestral stress responses, such as inflammation, and their pathways can be co-opted to create novel structural components. Examples of such innovations are rare, but include the extremely thick epidermis of cetaceans (whales and dolphins), and the decidual stromal cells of placental mammals. Another potential example is the so-called "plug" observed in Sulawesi ricefish (Beloniformes: Adrianichthyidae). The plug forms inside the female gonoduct after spawning, anchors egg-attaching filaments, and enables pelvic brooding species to carry eggs externally until hatching. We already showed that the transcriptome of the plug of *Oryzias eversi* is dominated by inflammatory signals and histological sections revealed that foreign body multinucleated giant cells (fused macrophages) are present. These are hallmarks of granulomas. Here we present first results of spatially resolved transcriptomics at four different time points during brooding. We aim to characterise the contribution of cell types underlying the cellular composition and the formation of the plug and to identify alterations in gene expression within the micro-anatomical structures of the reproductive system of *Oryzias eversi* females.

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## Experimental evolution of bacterial resistance is facilitated by niche construction: effects on microbiomes and antimicrobial secretions in red flour beetles

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Niche construction is a critical eco-evolutionary process by which organisms can modify their environment to enhance their adaptation to changing conditions. This process is especially relevant in group-living animals, as it aids in their defense against and adaptation to shared parasites. Despite its theoretical importance, empirical evidence remains scarce. Our ongoing evolution experiment with red flour beetles and their natural microparasite, *Bacillus thuringiensis tenebrionis* (Btt), uses RNAi to manipulate beetles to create differentially constructed niches (with and without stink gland secretions). Previous findings indicate that niches with secretions facilitate the evolution of pathogen resistance and influence transcriptomic responses, developmental programs, and early life fecundity. In this study, we analyzed changes in the microbiome via 16s RNA sequencing after 12 and 15 generations of selection. Additionally, we examined genetic changes in quinone secretion profiles using GC/FID after 18 generations. These results provide empirical evidence of the role of niche construction in facilitating evolutionary adaptation, and thereby highlight its significance in ecoevolutionary theory.

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### Rapid Rewiring: Experimental evolution of Spiroplasma in novel hosts

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Symbionts are integral to arthropod biology, profoundly shaping their hosts' traits and playing a pivotal role in speciation. While these associations can be costly for insect hosts, they are often stable and symbionts are maternally transmitted with high fidelity. However, the absence of co-cladogenesis between symbionts and hosts suggests that many such relationships are transient over evolutionary timescales, necessitating recurrent establishment in new/novel hosts via "host shifting." These host shifts are critical evolutionary events, influencing both host and symbiont evolution, but are poorly understood mechanistically. We used an experimental evolution approach to study the adaptation of the inherited symbiont Spiroplasma after host shifts. To this end, we artificially transferred Spiroplasma into novel Drosophila hosts species and passaged the symbiont in its novel hosts for 20 generations. We monitored Spiroplasma evolution through whole genome shotgun sequencing. We also determined the effect of Spiroplasma on Drosophila life history traits and how they evolve in response to Spiroplasma adaptation. Preliminary results indicate that the effect of Spiroplasma on host phenotypes depend on host and symbiont genetic backgrounds. Genomic analysis of adapted symbiont will provide insights into genetic changes underpinning these differences in phenotypes. These findings will elucidate how host shifting affects symbiont evolution in novel hosts, enhancing our understanding of the remarkable diversity and prevalence of inherited symbionts in arthropods. This research offers valuable insights into the evolutionary dynamics of hostsymbiont interactions, and will further illuminate the "Red Queen hypothesis" of rapid coevolution, where hosts and symbionts continuously counter-evolve.

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# Microbial genome dynamics: insights into the evolutionary transition to endosymbiosis

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Many insects carry heritable bacterial symbionts that play an important role in organismal function, ecology and evolution. However, the evolutionary processes and genomic adaptations associated with transitions to endosymbiosis and in particular vertical (parent to offspring) transmission are poorly understood. Here, we refine the current model of evolution during the early stages of the transition to vertically transmitted symbiosis. We used a comparative genomic analysis of the bacterial genus Arsenophonus (Gammaproteobacteria, Enterobacterales) to reveal the dynamic processes involved in the evolution of persistent symbiosis. We compared the genomes of several Arsenophonus strains with different life histories, ranging from environmentally acquired pathogenes to facultative and obligate insect endosymbionts. We show that the initial transition from environmentally acquired infection to persistent symbiosis is associated with a rapid genome and gene content expansion. This genome expansion is driven by the accumulation of phage and plasmid mobile elements enabled by the the loss of genome defence systems including CRISPR-Cas. This expansion of mobile genetic elements led to an expansion of T3SS effectors. The subsequent transition to an intracellular lifestyle and strictly vertical inheritance was associated with the expected relaxation of purifying selection, gene pseudogenisation, metabolic degradation and eventual genome reduction. Overall, our results suggest that the emergence of persistent endosymbiosis and vertical transmission requires rapid evolutionary innovation, which can be fueled by the horizontal acquisition of new traits necessary for host manipulation. This is enabled in part by the loss of genome defence systems and the subsequent invasion of mobile elements. Consequently, early-stage microbial symbionts may undergo rapid and extensive genome expansion prior to the processes of reductive evolution traditionally associated with adaptation to an intracellular lifestyle.

#### Drivers of sex chromosome turnover in the Lake Tanganyika cichlid radiation

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For many sexually reproducing species, genetic mechanisms of sex determination are essential for maintaining even sex ratios. Sex chromosomes are thought to represent the optimum genetic sex determining mechanism, by ensuring sex-specific inheritance of sexrelated traits. However, recent studies, particularly of fish, show that turnover of sex chromosomes is frequent, and can involve both turnover to a new chromosome, and to an alternative heterogamety pattern. This is principally demonstrated by the Lake Tanganyika cichlid fish radiation, in which these processes occur at the fastest rate known to date. Previous work has characterized sex chromosomes in almost a third of the 250 species of the radiation, but signals of sex-linkage in several species remain ambiguous. We reinvestigated signals of sex-linked genomic regions in 23 species at key proposed junctions in sex chromosome evolution of this radiation, using novel sequencing data and more precise methods of sex chromosome discovery. We were able to identify sex chromosomes of some species despite these being homomorphic and containing small sex-determining regions. Other species however showed no clear signal of sex-linked regions despite our highly sensitive methods, indicating that genetic sex determination has been repeatedly lost within these fish. We also show convergence on the same sex chromosome in divergent taxa, and investigate what ecological, reproductive or genomic traits may drive this. Finally, we identify potential drivers of heterogamety transitions that have occurred at the origin of some lineages. This reexamination of sex chromosome evolution in such a highly dynamic species group, reiterates the importance of high quality DNA data for research on sex chromosome evolution. Placing all of these factors influencing sex chromosome evolution in a phylogenetic context allows us to comprehensively uncover the essential drivers of sex chromosome evolution, turnover and loss, and provide stronger evidence that sex is an evolutionarily flexible trait.

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#### Phenotypic plasticity, epigenetics and the inheritance of non-genetic information

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Developmental plasticity and polyphenisms, the ability of a single genotype to form multiple phenotypes in response to environmental variation, have been proposed to represent a major facilitator of evolutionary divergence and novelty. While still contentious after a long time of neglect, the role of plasticity is gaining popular support through the development of various model systems that provide molecular and epigenetic insight into associated processes. Such studies are increasingly coupled with organismal work indicating ecological relevance and evolutionary significance of developmental plasticity. We will review the current understanding of mouth-form plasticity in the nematode Pristionchus pacificus that exhibits a mouth dimorphism with the eurystomatous (Eu) form being a potential predator on nematodes. whereas the stenostomatous (St) form is a strict bacterial feeder. After working on the genetic and epigenetic regulation of P. pacificus mouth-form plasticity for more than a decade, we recently started to use long-term environmental induction experiments to study the influence of shifts in microbial diet. Using a wild isolate of P. pacificus that is preferentially St on E. coli OP50, we performed long-term environmental induction experiments of 110 genetically identical lines for 101 generations on a Novosphingobium diet. We found immediate and systemic diet-induced plasticity, resulting exclusively in the formation of the Eu morph. Strikingly, periodic diet-reversals to OP50 starting in F15, F25 etc revealed transgenerational memory that entails multigenerational plasticity. We combined these long-term induction experiments with unbiased forward genetic screens and found a role of the ubiquitin ligase EBAX-1/ZSWIM8 in memory transmission. Subsequent studies revealed a repressive role of a microRNA cluster, the first demonstration of a role of microRNAs in transgenerational inheritance. We will review our current understanding of these processes and their potential role for evolution.

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## Unravelling genetic diversity of minnows (*Phoxinus*) from Germany: insights from historical stocking records, *COI*-barcodes, and whole-genomes

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Recent studies have revealed previously unknown cryptic diversity within Eurasian minnows (Leuciscidae - Phoxinus spp.), challenging its monotypic classification in Europe (Palandačić et al.,2015,2017). These findings have profound implications for conservation and taxonomy, particularly in countries like Germany, where multiple Phoxinus species coexist. The recognition of multiple genetically distinct lineages within *Phoxinus* led to a revision of German freshwater conservation laws to reflect the conservation needs of each recognised species (Freyhof et al., 2023). The existence of several species in Germany and the historical implementation of stocking and reintroduction programmes in the Middle and Lower Rhine prompted us to investigate the stocking sources and to this date unclear distribution of the "Rhine minnow" -P. phoxinus sensu stricto-, as well as the degree of differentiation and hybridisation between native and stocked Phoxinus species. Stakeholder (individual fishing right holders, angling clubs, administrative authorities) surveys and literature review covering the last 150 years revealed stocking of non-native *Phoxinus* in the Sieg drainage, a tributary to the Lower Rhine, corresponding also to the type locality of *P. phoxinus* (Kottelat, 2007), which is further supported by whole-genome analyses results. Whole-genome analyses of over 300 individuals from local (Germany) and in surveys identified stocking sources (Germany, France) further revealed genetically distinct populations in drainages of Meuse, Seine (both P. phoxinus), and Rhône ("French minnow", P. septimaniae). Notably, Seine and Meuse P. phoxinus share the same COI-clade identity, 10, but appear as unique evolutionary significant units (ESU). In the Middle and Upper Rhine, another distinct ESU of the Danube minnow P. csikii (corresponding to COI-clade 5b) is found, hereafter called Rhine-type P. csikii. The Lower/Middle Rhine boundary might actually be home to both-the previously unknown Rhinetype P. csikii as well as to the P. phoxinus also found in the Meuse. These findings underscore the significance of whole-genome analyses in conservation, aligning with indicators proposed by the Global Biodiversity Framework (GBF) for identifying within-species diversity and genetically distinct populations (Hoban et al. 2020, Laikre et al. 2020).

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#### Novel genomic exons: adaptation over evolutionary time and genomic space

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Genomic and transcriptional plasticity is significantly shaped by newly integrated genetic components, along with mobile genetic elements such as transposons. Many of these elements are eventually lost, but some can impact host genomes for extended periods through processes such as exonization and possible "domestication." and exaptation. Although exonization and its underlying mechanisms have been investigated previously, much of this research has focused on a limited subset of novel genetic elements and specific subgroups within them. Using our extensive database of over 1100 documented cases of transposon exonizations in the human genome (most of which were hitherto undescribed), we set out to explore the evolutionary consequences and molecular mechanisms associated with the emergence, subsequent loss, or exaptation of these novel genetic elements. We employed large-scale comparative selection analyses of the exonized regions and their surrounding coding sequences, streamlined and facilitated by custom software tools such as paPAML and NewickTreeModifier. Additionally, we incorporated transcriptome data and protein structure analyses into our investigation.

#### Optimal division asymmetry in aging bacteria

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Asymmetry at cell fission is thought to be the origine of division of labour, of multicellularity, and of the evolution of aging. In Escherichia coli bacteria such asymmetries between the aging mother and their daughter cells manifest in growth differences, recently discovered morphological differences in size, and protein distributions—including protein clusters and other transcription and translation related processes that lead to unequal partitioning of cytoplasm content. Here we explore, for growth and a general stress response regulator (rpoS), whether the observed mother-daughter asymmetry is optimal with respect to population fitness. We do this by structured population modelling for which models are parameterized with high throughput single cell data and explore deviations of the observed asymmetry for population growth. Our findings illustrate that increased or decreased asymmetries in size and rpoS lead to reduced population growth, suggesting that evolution might have optimized the observed levels of asymmetries. We further show that variability in size or rpoS expression dynamics, as well as altered covariance between size and rpoS expression influence fitness only to a minor degree. Combined this suggest that the observed asymmetries at cell division likely have evolved adaptively but that variance around these asymmetries are under little selection. As asymmetries foster heterogeneity among cells, and such heterogeneity can influence evolutionary dynamics—one example being phenotypic resistance to antibiotics—it is of interest to explore the evolution and maintenance of such non-genetic heterogeneity, an area where to date little understanding exists.

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### Species sorting among two species of *Phoxinus* (Leuciscidae) in their natural contact zone - the Sieg basin (Rhine)

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Eurasian minnows of the genus Phoxinus (Leuciscidae) are a prominent case of cryptic diversity. This is evident between the genetically distinct yet morphologically similar Phoxinus phoxinus and P. csikii. In the Sieg system (right-sided Rhine tributary near Bonn, Germany), the two species exhibit a natural contact zone in their distribution. While P. csikii inhabits the mountainous regions in the Sieg system, P. phoxinus prevails in the foothill regions of the basin. The distribution of the two species might thus represent a case of species sorting. In the metacommunity concept, the species-sorting perspective emphasises that variation in community composition is driven by environmental variation among discrete habitats. Therefore, a key role influencing the distribution of the two *Phoxinus* species might be their specific niche preferences. To understand factors promoting species sorting, an extensive sampling of *Phoxinus* populations in the Sieg basin was implemented over two years (two summer and two spring field seasons, each). Subsequently, we focussed on the diet and trophic niche, as well as habitat preferences of the two *Phoxinus* species, by employing stable isotope analysis, stomach content analysis, and ecological niche analysis. Preliminary results show evidence for different trophic niches and habitat preferences between both species. P. csikii was characterised by a higher trophic position and wider trophic niche, but a smaller habitat niche breadth than P. phoxinus. These findings support the hypothesis that species sorting is the driving force of the distribution of *P. csikii* and *P. phoxinus* in the Sieg basin.

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### Genetic architecture of pheromone variation and prezygotic isolation in two parasitoid wasp species

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One of the most important questions in biology is how new species originate. Variations in sexual signals can contribute greatly to reproductive isolation between species by enabling species-specific communication and recognition. Cuticular hydrocarbons (CHCs), major recognition molecules on the cuticle of insects, have been shown to encode species-specific sexual signals in many taxa. However, there is still limited knowledge on which specific compounds in complex CHC profiles mediate species-specific mate choice. Similarly, the genetic basis governing CHC variations involved in reproductive isolation remains largely elusive so far. The present study closes these knowledge gaps in characterizing the chemical and genetic basis of species-specific CHC variation and its consequences for prezygotic reproductive isolation between two closely related parasitoid wasp species. Exploiting their haplo-diploid sex determination and cross-fertility under laboratory conditions, we generated female recombinant inbred lines (RIL), constituting both genetic and phenotypic hybrids of the parental species. We conducted quantitative trait loci (QTL) analyses of all individually identified CHC compounds separately for the diploid female recombinant lines as well as their haploid male fathers. Correlating CHC quantities with mate preferences, we identified three key compounds significantly correlated with male mating behavior frequencies. Further zooming into the QTL for the species-specific variation of our three key pheromonal compounds, we were able to localize several CHC biosynthesis candidate genes. Further knockdown of one of the candidate genes largely impacted the newly identified key compounds as well as structurally similar CHCs. Thus, through identifying the species-specific key components out of complex pheromonal blends mediating mate preference, we determined the molecular and chemical basis of a prezygotic isolation mechanism.

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#### Impact of multigenerational inbreeding on sexual selection in a cichlid fish

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Animal mating systems are assumed to be characterized by outbreeding and active inbreeding avoidance. However, recent studies suggest that inbreeding avoidance is rarer than expected and theory predicts fitness benefits from inbreeding. Pelvicachromis taeniatus, a biparental cichlid fish from West Africa has been shown to prefer kin as mating partners. Inbreeding is adaptive because related parents are more cooperative during brood care, while we did not find evidence for inbreeding depression in F1-inbred offspring. Here, we examined the impact of multigenerational inbreeding on kin-mating preference, mate choice, attractiveness and intra-sexual competition. Continuous inbreeding (resulting from full-sibling mating) altered kin preferences. While outbred fish showed similar kin preferences as reported in previous studies, fourth-generation inbred fish preferentially mated with non-kin. This generation also revealed signs of inbreeding depression at early developmental stage. However, offspring resulting from crosses between inbred lineages regained fitness suggesting genetic rescue. Furthermore, highly inbred adult fish were less selective during mate choice with respect to the phenotypic quality of potential partners, less attractive as partners and poorer competitors over breeding sites. Our results suggest that the costs of inbreeding are not constant but change with persisting inbreeding. Kin-mating preferences seem to oscillate according to the associated costs and benefits. We discuss the consequences of population structure for the evolution of inbreeding avoidance and preference.

#### The coffee berry borer, Hypothenemus hampei: Resilience to extreme heat waves

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Global warming leads to higher average temperatures and more frequent heat waves. Here, we report how temperature affects a major coffee pest, the coffee berry borer (CBB) *Hypothenemus hampei* (Ferrari). Using artificial habitats, the lethal temperature was found to be at 44°C. Subsequently, females were exposed to temperatures ranging from 36°C to 43°C. for four hours per day for three days, after which they were kept at 27°C (21°C at night) for seven weeks. During these seven weeks, the beetles were monitored weekly with tunnel digging activity assessed in week 1, fecundity in week 5 and sex ratio in week 7. Additionally, heat wave effects on bacterial endosymbionts were investigated via qPCR. Overall, our results show that exposure to 43°C heat waves resulted in lower fecundity when compared to the other temperatures. It should be taken into account that the mortality at 43°C was high with 74% of females dying within the first two weeks. Furthermore, sex ratios remained unaffected regardless of temperature. We found that digging activity was highest when beetles were exposed to 41°C heat waves, and that heat waves from 36°C to 43°C had no on influence the bacterial titers of CBB females. These results indicate that the CBB can withstand heat waves up to its lethal limit, with only minor consequences for reproduction.

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## Population and Evolutionary Implications of Stochastic and Asymmetric Cellular Damage in Bacteria

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Ageing disrupts cellular integrity and affects fitness components such as reproduction and survival. The characteristics of ageing influence not only population growth rates (i.e., population fitness) but also evolutionary dynamics, as the fate of new mutations is shaped by individual ageing profiles. Remarkably, even within a genetically and environmentally uniform population, substantial variation in ageing characteristics, such as lifespans, can be observed. However, this stochastic variation has often been overlooked. In our research, we focus on understanding the molecular and cellular basis of stochastic variation in ageing and its evolutionary consequences. We utilise microfluidic devices combined with fluorescent microscopy to track individual E. coli cells, capturing data on gene expression, cell physiology, and mortality. In this talk, I will present our work scaling the demographic patterns of individual E. coli cells observed through microscopy to population-level growth characteristics in batch cultures under glucose-limitation stress conditions. Interestingly, we find that this stress affects the mortality of single-cell lineages, while the reproduction (cell division) rates remain relatively stable unless the cells die or become dormant. Using a cellular damage model and its projection to population dynamics, we show that the observed differences in single-cell mortality and division rates align with the observed decline in population-level growth under this glucose-limitation stress. In the final part of the talk, I will discuss how this theoretical model might help us explore how molecular features of damage, such as damage accumulation noise and damage partitioning asymmetry during cell division, influence population growth (i.e., population fitness) and how these processes may be shaped by evolutionary forces.

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#### How does mate limitation affect evolutionary rescue for different mating systems?

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Environmental change may lead to drastic declines in the population size and drive populations towards extinction. For populations with biparental sexual reproduction, the decline in the population density may entail another risk in addition to the external stress: a reduced probability to find a mate. Mate limitation may constrain successful adaptation to the new environment and increase the risk of population extinction. Nevertheless, most models of evolu- tionary rescue assume that mating is assured even at small population sizes. In this talk, I present results from mathematical modeling for rescue under mate limitation. We consider populations with three different mating systems which differ in their reproductive pools and mate pools and thus in the intrinsic extent of mate limitation: dioecious, hermaphroditic, and androdioecious populations. In addition, we vary how sensitive the mate finding probability is to changes in the population size. As expected, for all three mating systems, the probability of rescue is lower when reductions in the population size make failure to find a mate more likely. We furthermore find that for dioecious populations, the sex ratio that maximizes rescue shifts from female-biased to a 1:1 ratio with in- creasing mate limitation. Overall, our results demonstrate the need to account both for the specificities of the mating system and for the density dependence of mate finding for the assessment of a population's scope for evolutionary rescue.

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#### Phenotypic robustness differs between queen and worker caste

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In multicellular organisms, the germline is preserved with high fidelity across generations, safeguarded from environmental variation to maintain genetic continuity. Analogously, in social insect colonies, the gueen serves as the protected reproductive unit, ensuring the stability of the colony's lineage while the worker caste adapts to fluctuating ecological demands. This parallel raises the question whether queens, like the germline, exhibit enhanced phenotypic robustness, especially under stress, compared to the more susceptible worker caste. Therefore, we tested whether phenotypic robustness differs between castes by quantifying the degree of morphological and transcriptomic variation between gueens and workers of the ant Cardiocondyla obscurior upon pharmacological suppression of Hsp90, a central player of the cellular canalization machinery. Our findings show that Hsp90 inhibition leads to an increased variation in workers, but not in queens, both on the phenotypic and transcriptomic level. Therefore, analogous to the germ-plasm theory, queens (germline) seem to show higher phenotypic robustness under stressful environmental conditions. Based on in-depth transcriptomic analyses, we conclude that queen phenotypic robustness is realized through steady expression of specific gene sets even under Hsp90 inhibition, potentially revealing caste-specific molecular strategies for maintaining phenotypic robustness of queens, securing the reproductive and generational integrity of the colony.

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## A comprehensive representation of selection at loci with multiple alleles that allows complex forms of genotypic fitness

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Genetic diversity is central to the process of evolution. Both natural selection and random genetic drift are influenced by the level of genetic diversity of a population; selection acts on diversity while drift samples from it. At a given locus in a diploid population, each individual carries only two alleles, but the population as a whole can possess a much larger number of alleles, with the upper limit constrained by twice the population size. This allows for many possible types of homozygotes and heterozygotes. Moreover, there are biologically important loci, for example those related to the MHC complex, the ABO blood types, and cystic fibrosis, that exhibit a large number of alleles. Despite this, much of population genetic theory, and data analysis, are limited to considering biallelic loci. However, to the present, what is lacking is a flexible expression for the force of selection that allows an arbitrary number of alleles (and hence an arbitrary number of heterozygotes), along with a variety of forms of fitness. In this work, we remedy this absence by giving an analytical representation of the force of selection that emphasises the very different roles played by the diversity of the population, and the fitnesses of different genotypes. The result presented facilitates our understanding and applies in a variety of different situations involving multiple alleles. This includes situations where fitnesses are: additive, multiplicative, randomly fluctuating, frequency-dependent, and it allows fitnesses which involve explicit gene interactions, such as heterozygote advantage.

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### Transcriptomic adaptations in *Aphidius ervi*: Disentangling genetic and plastic responses in host- parasite evolution

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Parasites are competing in an endless evolutionary arms race with their hosts, continuously undergoing adaptations. Such adaptations can be realized through genetic adaptations or phenotypic plasticity. Disentangling the contribution of plasticity and genetic adaptation requires the exclusion of either factor to study the other. In this study, we established two highly inbred, and therefore genetically homogenous strains (families) of the parasitoid wasp *Aphidius ervi* to explore the capacity for adaptive plastic responses to different hosts. Furthermore, we explored whether > 20 generations of specialization towards a specific host leads to heritable phenotypic or transcriptomic responses not embedded in genetic adaptation. We used RNA-Seq and morphometrics to characterize the transcriptomic and phenotypic responses to the respective hosts at different levels of interaction. Our results suggest highly plastic responses of both, phenotype and transcriptome, to contrasting hosts, but very little effects of previous host experience. In addition, we found strong transcriptomic differences between families, emphasizing the role of genetic drift in driving neutral divergence of gene expression.

#### When the Past Fades: Detecting Phylogenetic Signal

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In phylogenetics, the phenomenon of saturation is well known, although its influence on tree reconstruction lacks a systematic and well-founded method. Here we propose a new measure of the phylogenetic information shared between two subtrees connected by a branch in a phylogeny. This measure generalises the concept of saturation between two sequences to a theory of saturation between subtrees, whose implementation we provide as the versatile program SatuTe. We describe different usages of SatuTe, identifying which branches in a tree are phylogenetically informative and which alignment regions support a given branch. As an example, we discuss the Tree of Life reconstruction from ribosomal proteins and the 16S rRNA gene, with emphasis on the two-domain versus three-domain hypotheses. For the branch leading to Eukaryota, we show that most ribosomal proteins contain a strong phylogenetic signal, whereas some regions of the 16S rRNA gene have lost phylogenetic information. Our method opens new insights into phylogenetic inference and complements standard phylogenetic analysis.

#### Competition during co-infection drives the evolution of integrative viruses

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Integrative viruses, such as lambdoid prophages, integrate into the chromosome of their host at specific insertion sites via site-specific recombination. Individual E. coli strains can harbor multiple prophages at different chromosomal positions. However, the variation in prophage types occupying the same insertion sites across different hosts suggests that some prophages compete for the same site. Despite this observation, the mechanisms underlying how integrative viruses resolve this competition and its evolutionary consequences remain poorly understood. Drawing parallels to ecological niche competition, where during co-infection, different viruses compete for specific insertion sites, we hypothesized that following the principle of competitive exclusion, a virus that loses the competition must either find a new position or fail to persist. To investigate whether competition for insertion sites drives the diversification of novel sites, we conducted serial transfer experiments in which different lambdoid phages competed for the same site. Our findings reveal that in the absence of competition, phages dominate the population by forming lysogens, each following phagespecific trajectories determined by phage-specific fitness costs. Conversely, in the presence of competition, most bacteria acquire one or no prophage, often accompanied by phageresistant mutations for non-acquired phages. Only a small fraction of the population acquired both phages, where integration into novel positions is facilitated by changes in the viral genome. These phages exhibited reduced fitness compared to when they integrate in the absence of competition, suggesting that escaping competitive exclusion may result in maladaptation to the alternative niche, decreasing the fitness of both the host and the virus. Our findings emphasize the importance of ecological competition in driving viral diversity and host evolution, with broad implications for understanding host-virus co-evolution. Competitive exclusion and niche adaptation appear critical in viral evolution, potentially leading to the emergence of new viral strains with unique genomic characteristics.

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## MHC diversity has a sex-dependent influence on fitness in a social mammal, the banded mongoose

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Pathogen infections affect males and females differently across a wide range of species, often due to differences in immune responses. Generally, females tend to have stronger immune defenses and lower parasite loads than males. The major histocompatibility complex (MHC) plays a crucial role in the adaptive immune response, and extensive research has explored how variation in this region influences infection and fitness outcomes. However, studies investigating sex-specific relationships between MHC variation and infection are scarce, perhaps because the MHC genes are located on nuclear chromosomes shared by both sexes. In this study, we provide evidence of sexually antagonistic selection in a wild, group-living mammal—the banded mongoose. Using genetic and life history data collected from over 300 individuals across 25 years, we found that MHC class I (MHC-I) diversity influences lifetime reproductive success differently in males and females. Specifically, higher MHC-I diversity is linked to increased fitness in males but decreased fitness in females Interestingly, MHC diversity did not differ between the sexes, indicating an unresolved genetic sexual conflict. In contrast to MHC-I, there was little evidence for an impact of MHC-II diversity on fitness in our study population. Our findings demonstrate that sexually antagonistic selection acts on the MHC and may operate differently across MHC classes. This study contributes to the growing body of evidence that sex is a significant factor in shaping host immunity and fitness.

#### Genetic regulation of complex social behaviors in male mating morphs

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Intense competition over matings has led to the evolution of elaborate ornaments and behaviors in males, allowing dominant individuals to secure most matings. Occasionally, subdominant males adopt alternative reproductive tactics to achieve reproductive success. These tactics are often associated with distinct physiological adaptations, such as altered concentrations of sex steroids, which are thought to underlie the behavioral differences. However, how variation in sex steroid hormone levels affects the brain, and thus behaviors, remains unclear. In this study, we analyzed transcriptomic data to profile gene expression variation across nine brain regions of different neural circuits in a lekking sandpiper, the ruff (Calidris pugnax). This species features three distinct mating morphs, determined by a supergene containing around 100 genes. During the breeding season, males of these morphs show near-discrete variation in aggression and courtship behaviors, as well as in circulating androgen concentrations. Two non-aggressive morphs, Satellites and Faeders, are characterized by low circulating testosterone whereas males of most common morph, Independents, have high circulating testosterone and exhibit territorial aggressive behaviors. At the same time, Satellites and Independents perform elaborate courtship displays, while Faeders mimic females in appearance and behavior to sneak copulations. Here, we capitalized on the existing natural genetic and behavioral contrasts between ruff mating morphs to investigate the proximate mechanisms of social behaviors in this species. Using a combination of differential gene expression and gene co-expression network analyses, we identified and characterized sets of candidate genes underlying aggression and courtship in the brains of the three ruff morphs. Taken together, these results provide unique insights into the link between gene expression variation and the evolution of elaborated social behaviors.

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