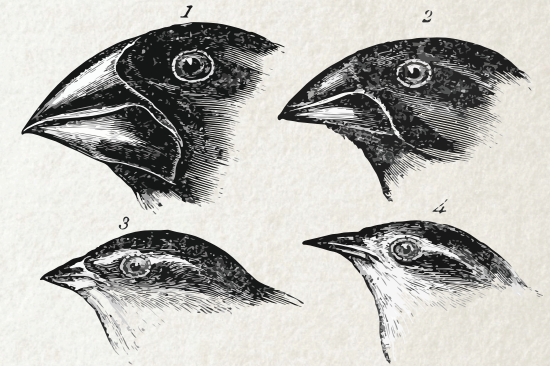
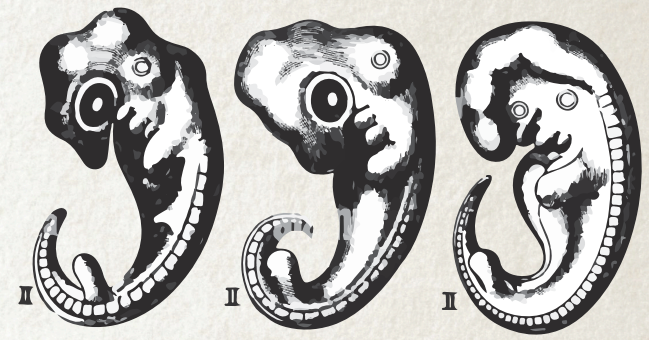


# Concepts in Evolutionary Biology

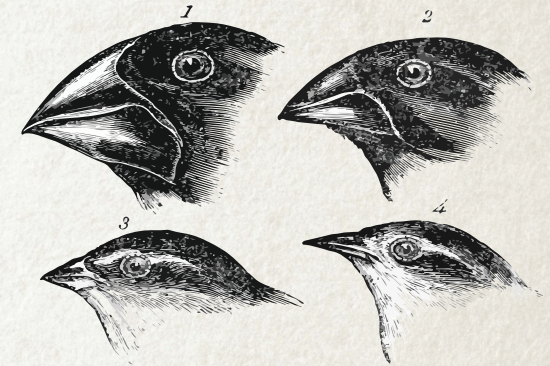
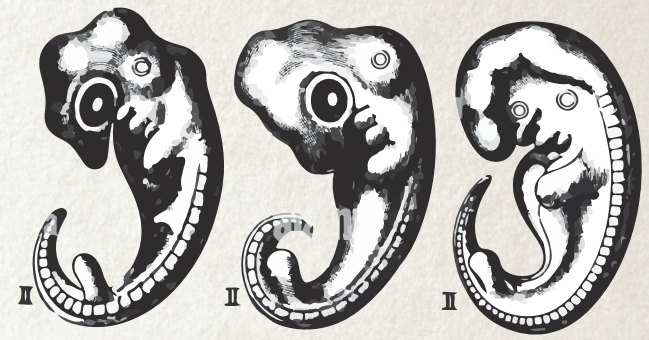


**10- 13<sup>th</sup> FEBRUARY 2025**

**PLÖN, GERMANY**

**Max Planck Institute for  
Evolutionary Biology**

# Concepts in Evolutionary Biology



**10- 13<sup>th</sup> FEBRUARY 2025**

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# Welcome note



Evolutionary Biology as a field has undergone several transitions. From publication of *On the Origin of Species*, through holistic gradualism, reconciliation with genetics and the emergence of population genetics, recognition of the role of epigenetics and development, multilevel selection, to predicting evolution. These reflect not only changes in the approaches for addressing evolutionary questions, but also in factors considered relevant and explanatory for evolutionary phenomena.

The aim of the workshop is to discuss fundamental concepts in evolutionary biology and thus ensure that new generations of evolutionary biologist possess appropriate toolkits of ideas. The format is as follows

## **Concept and Empirical talks**

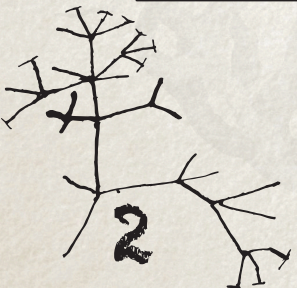
Sessions are organized by themes. Invited speakers are requested to present a chalk talk, where a key concept is presented and argued (40 min + 20 min Q&A). Following this a second invited speaker will present empirical data compatible with the session topic (40 min + 20 min Q&A).

## **Open discussions**

Each day there will be 1 hour long sessions reserved for people to split in groups (three rooms: Lecture Hall, practical room and Cafeteria) and discuss topics of their preference. Topics are open for people to propose and choose to attend.

## **Poster sessions**

Current research of participants can be presented in the form of posters. Poster sessions will be in the evening, in a relaxed and informal setting.



# Monday 10th

15:00 → 16:20

**Announcements**

Registration and Check-in (1h)

Welcome words (20min)

16:20 → 17:20

**Niche Construction**

Concept Talk:

Dr. Kevin Lala

17:20 → 17:30

**Short Break**

17:30 → 18:30

**Niche Construction**

Empirical talk:

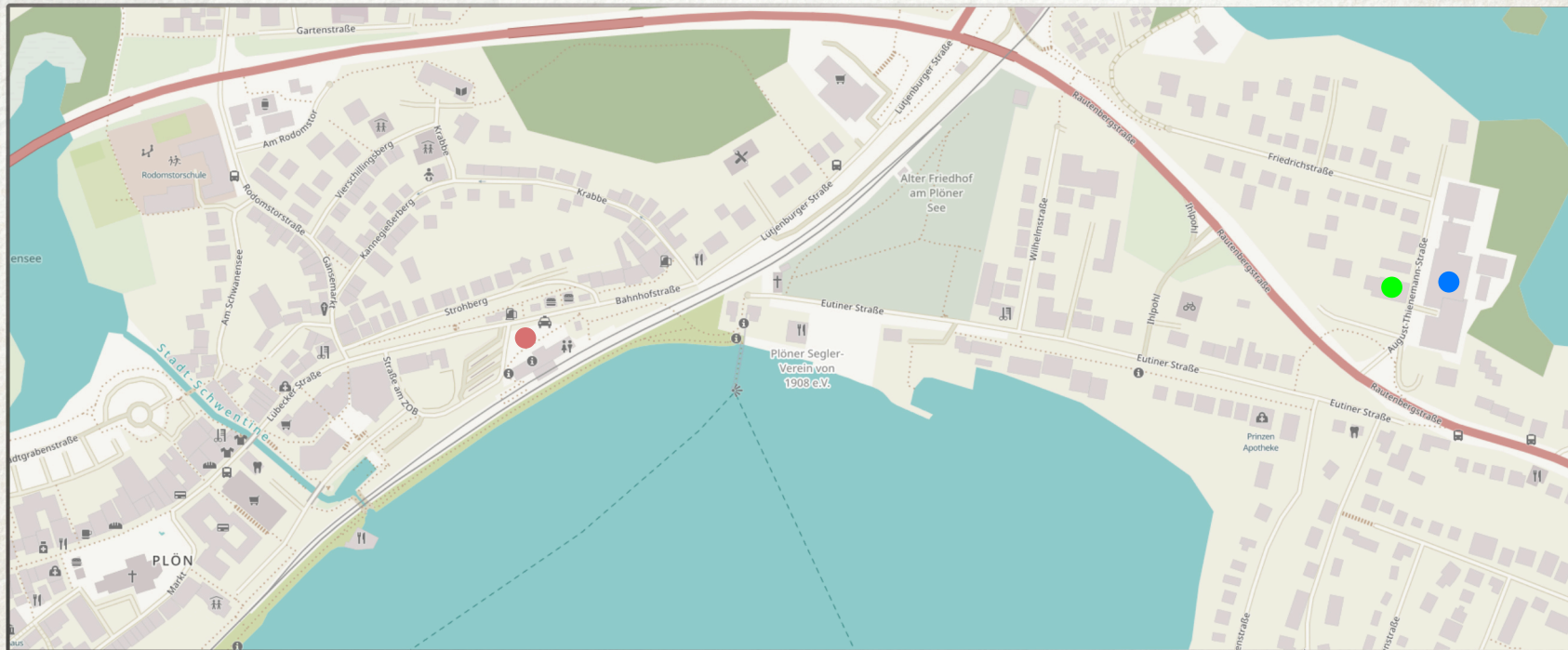
Dr. Blake Matthews

18:30 → 19:30

**Dinner**

19:30 → 20:30

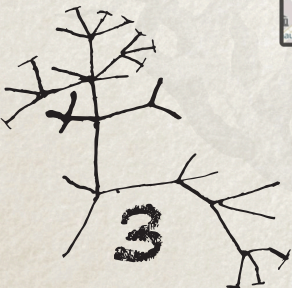
**Poster Session 1**



● Train station

● Lecture Hall

● Institute



# Tuesday 11th

9:00 → 10:00 **Early replicators and origin of life**

Concept Talk:

Dr. Nick Lane

15:50 → 16:20

**Coffee Break**

16:20 → 17:20

**Holobiont and HGT**

Empirical talk:

Dr. Honour McCann

10:00 → 10:30 **Coffee Break**

10:30 → 11:30 **Evolutionary developmental biology**

Concept Talk:

Dr. Nathalie Feiner

17:20 → 17:30

**Short Break**

17:30 → 18:30

Discussion day 1

11:30 → 11:40 **Short Break**

11:40 → 12:40 **Evolutionary developmental biology**

Empirical talk:

Dr. Emilia Santos

18:30 → 19:30

**Dinner**

19:30 → 20:30

Poster Session 2

12:30 → 13:40 **Lunch**

13:40 → 14:40 **Evolutionary developmental biology**

Empirical talk:

Dr. Markéta Kaucká

14:40 → 14:50 **Short Break**

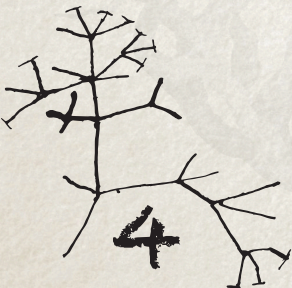
14:50 → 15:50 **Holobiont and HGT**

Concept Talk:

Dr. Ford Doolittle



Photo: Michael Hesse



# Wednesday 12th

9:00 → 10:00 **Fitness landscapes**  
Concept Talk:  
Dr. Justin R. Meyer

10:00 → 10:30 **Coffee Break**  
10:30 → 11:30 **Fitness landscapes**  
Empirical talk:  
Dr. Andreas Wagner

11:30 → 11:40 **Short Break**  
11:40 → 12:40 **Adaptive Radiations**  
Concept talk:  
Dr. Dolph Schluter

12:30 → 13:40 **Lunch**  
13:40 → 14:40 **Adaptive Radiations**  
Empirical talk:  
Dr. Christopher Martin

14:40 → 14:50 **Short Break**  
14:50 → 15:50 **Predicting evolution**  
Concept Talk:  
Dr. Meike Wortel

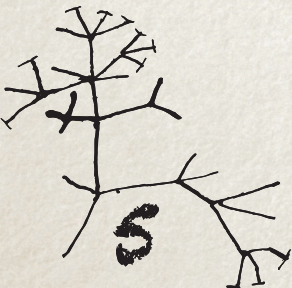
15:50 → 16:20 **Coffee Break**  
16:20 → 17:20 **Predicting evolution**  
Empirical talk:  
Dr. Peter Lind

17:20 → 17:30 **Short Break**  
17:30 → 18:30 **Discussion day 2**

18:30 → 19:30 **Dinner**  
19:30 → 20:30 **Poster Session 3**



Photo: Michael Hesse



# Thursday 13th

9:00 → 10:00 **Multilevel selection**

Concept talk:

Dr. Paul Rainey

10:00 → 10:30 **Coffee Break**

10:30 → 11:30 **Multilevel selection**

Empirical talk:

Dr. Katrin Hammerschmidt

11:30 → 11:40 **Short Break**

11:40 → 12:40 **Polygenic adaptations**

Concept Talk:

Dr. Marcus Feldman

13:40 → 14:40 **Polygenic adaptations**

Empirical talk:

Dr. Neda Barghi

14:40 → 14:50 **Short break**

14:50 → 15:50

Discussion day 3

15:50 → 16:10

Closing remarks

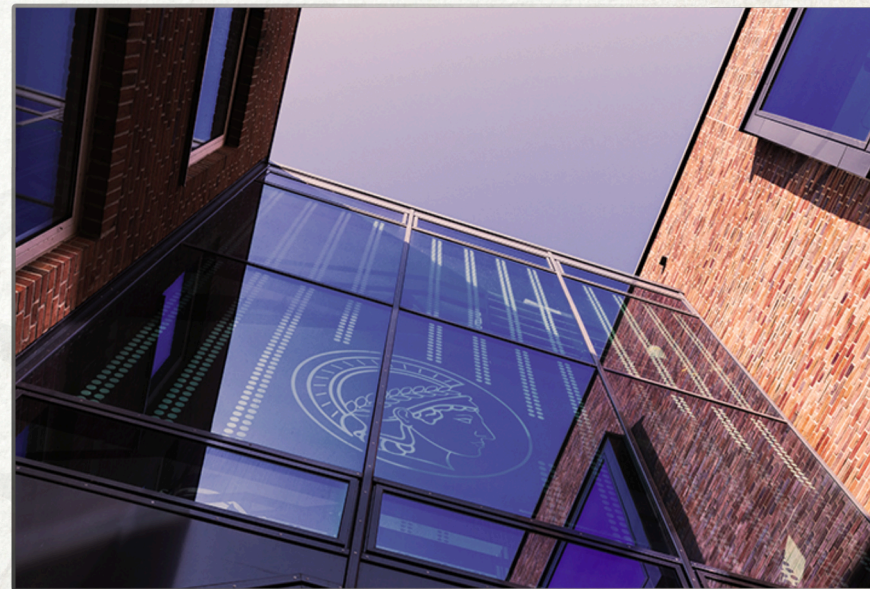


Photo: Michael Hesse



# Posters

## Single-cell phylotranscriptomics reveals developmental hourglass pattern of cell lineages

Amor Damatac II, Kristian Ullrich, Markéta Kaucká

MPI for Evolutionary Biology

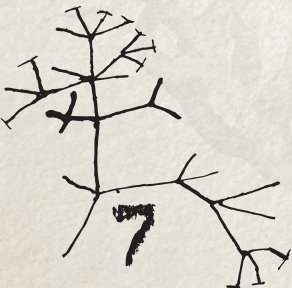
The evolutionary history of the molecular program of cell states can be inferred by tracing the evolutionary origin of the genes they express. In this study, we employ a single-cell phylotranscriptomic approach, combining gene birthdating and single-cell transcriptomics, to investigate the existence of the developmental hourglass pattern at the cellular level. We reconstructed the cellular trajectories during vertebrate embryogenesis to elucidate the shifts in the evolutionary age of cell state transcriptomes across developmental stages.

## Spatiotemporal profiling and molecular characterization of cranial neural crest cells in the small-spotted catshark

Elio Escamilla-Vega, Andrea P. Murillo-Rincón, Louk W.G. Seton, Ann-Katrin Koch, Timo Moritz, Markéta Kaucká

Evolutionary Developmental Dynamics RG, Max Planck Institute for Evolutionary Biology

Neural crest cells (NCCs) are vertebrate-specific multipotent cells arising during neurulation. NCCs migrate to different locations in the body, where they proliferate and give rise to numerous cell types and tissues. Due to their capacity to form, among other cell types, pigment cells, chondrocytes and osteocytes, the NCCs contribute to the formation of species-specific and individual-specific morphological traits such as colouration and geometry of skeletal elements. The evolution of NCCs substantially contributed to species diversification, enhancing the interest in understanding their species-specific biology. The main body of neural crest research has been carried out on model organisms such as the mouse, chicken and zebrafish. However, to understand the evolution of NCCs and NCCs-related traits, it is required to comprehend their molecular underpinnings and other properties on animals across the vertebrate phylogeny. Here, we characterize the molecular profile of cranial NCCs in the small-spotted catshark (*Scyliorhinus canicula*), a representative of phylogenetic node Chondrichthyes (cartilaginous fish). We present a comprehensive molecular profile and behaviour of cranial neural crest cells, employing 3D imaging, fate mapping experiments and single-cell transcriptomic. This knowledge will be fundamental to future comparative studies that will dissect the effect of NCC evolution on the formation of distinct morphologies.





# Posters

## RNAseq analysis reveals extensive within- and across-generation transcriptional plasticity to changes in food quality

Karem Lopez-Hervasand<sup>1</sup>, Anja Guenther

Max-Planck Institute for Evolutionary Biology

When facing environment change, animals need to adjust to survive and reproduce. These adjustments are often plastic, occurring fast as flexible responses during an animal's lifespan or may be induced by parental effects intergenerationally. Here, we use a transcriptomic approach to explore the mechanistic basis of immediate and intergenerational plasticity in response to changes in food quality. We conducted a full factorial match-mismatch experiment in semi-naturally living house mice populations to investigate within-generation and intergenerational responses to changes in food-quality from better to worse and vice versa. Mice populations differ in behaviour, life-history and transcriptomic profiles depending on the food-quality regime. As an immediate response to a shift away from high-quality conditions, animals showed a strong but mostly transient response, already showing first changes in expression patterns towards the new optimum. When switching away from worse conditions (to better conditions), however, mice showed an immediate, strong but unspecific stress-response and changed expression patterns of metabolically active genes further away from the new optimum, indicating condition-dependent flexibility that buffers individuals originating from high-quality environments against environmental stress. One generation after the change, gene expression adjustment aligned more closely with adaptive expectations and both treatments showed intermediate expression profiles. Full adaptive adjustment to a new environment in terms of gene expression, life-history, and behaviour—requires more than one generation for mice. Thus, while plasticity is undoubtedly important to mitigate fast and unpredictable environmental change, adaptive transgenerational effects may often only be detectable several generations after the environment changed.

## Emergence of increased heredity by selection on a collective trait

J. Carlos R. Hernandez-Beltran<sup>1</sup>, Ellen McConnell<sup>1</sup>, Inga Schmidt<sup>1</sup>, David W. Rogers<sup>1</sup>, and Paul B. Rainey

Max-Planck Institute for Evolutionary Biology

The study of evolution has traditionally focused on the effects of selection on individuals. In recent years, attention has turned to the possibility that selection might work on communities, but much remains to be understood about necessary conditions and consequences. Previous theoretical work has shown that communities can evolve as units of selection provided the communities themselves are endowed with Darwinian properties. In the laboratory, the experimenter can readily ensure discreteness of communities and thus variation among the units. Reproduction can be effected by taking multiple samples from a given community and using these to found offspring communities. What is undefined is the basis of the community-level parent-offspring relationship. In the absence of interactions, this is governed by stochastic sampling effects. Our interest is in the evolution of interactions that improve the parent-offspring relationship and thus underpin community-level heredity. Here, we describe the results of a proof-of-principle experiment: communities comprised of two populations of self-replicating plasmids (individuals) nested within single replicating yeast cells (collectives). We use two 2 $\mu$  plasmids that differ only in the fluorescent reporter they carry (either green or red). As the plasmids share the same origin of replication, each cell may end up with different proportions of green and red plasmids, thus producing a green-yellow-red phenotype spectrum. After 70 rounds of community-level selection on  $\sim 1.5 \times 10^8$  communities, with selection for yellow cells (requiring a balance between green and red plasmids), not only were we able to maintain the collective phenotype but the fraction of cells expressing it increased over time, indicating enhanced heredity levels and suggesting evolution at the level of individuals. When selection was removed on the evolved communities, they maintained the selected phenotype, indicating endogenization of the target character. This endogenization entails an evolutionary transition into individuality. Furthermore, derived communities were resilient against counter-selection for single individual types. Sequencing of the evolved collectives showed that the plasmids had merged into a new type of individual capable of producing the objective community phenotype.



# Posters

## (Epi)genetic control of meiotic recombination rate evolution in mice and humans

Rajalekshmi.N, Nicole Thomsen, Dr. Linda Odenthal-Hesse<sup>1</sup>

Max-Planck Institute for Evolutionary Biology

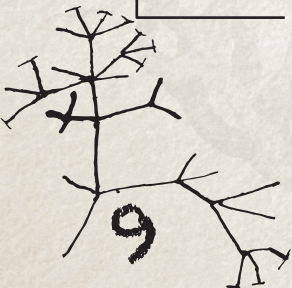
Meiotic recombination ensures proper segregation of homologous chromosomes, essential for fertility and avoiding aneuploidy. It also introduces genetic variation, characteristic to sexual reproduction. The rate of recombination varies across species, individuals, sexes, chromosomes, and even regions within a chromosome. In mice and humans, PRDM9 determines the non-random sites of recombination using DNA-specific binding and epigenetic histone methylation. PRDM9 is the only known hybrid sterility gene in vertebrates so far. However, hybrid sterility is an oligogenic trait. The extended hybrid sterility locus on the mouse X-chromosome houses a family of microRNAs called the SpermiRs, implicated in male sterility, are candidate recombination regulators. We study the microRNA-based epigenetic post-transcriptional regulation of recombination rate which are working in tandem with other well-studied genetic regulators of recombination in mammals. We characterize the within-species variation, the birth, death, expansion and evolution of the candidate epigenetic meiotic recombination regulators in mice and humans in terms of hybrid male sterility. We wish to test the impact of natural alleles of (epi)genetic factors involved in recombination on the meiotic transcriptome, focusing on how variation in the microRNA lead to variation in the mRNA targets. We envision a comprehensive evolutionary description of the nature and dynamics of the putative epigenetic regulators of meiotic recombination in the context of diversity, hybrid sterility, and speciation.

## Circalunar clocks as examples of evolutionary tinkering and convergent evolution

Jule Neumann , Danila Voronov , Dharanish Rajendra , Tobias S. Kaiser

Max-Planck Institute for Evolutionary Biology

Organisms have evolved endogenous, molecular timekeeping mechanisms – so called biological clocks – to time and coordinate essential life processes. The circadian clock, which controls the timing of an organism's daily business, can be found from bacteria to humans and is well understood at the molecular level. Circatidal clocks (i.e. anticipating the tides) and circalunar clocks (i.e. anticipating lunar phases) are wide-spread in marine organisms, but their molecular basis remains enigmatic. In recent years, evidence has accumulated that the circalunar clock of the marine midge *Clunio marinus* is likely derived from a photoperiodic diapause mechanism. First, it is known that the circalunar clock is synchronized by light at night, involving a sensitivity window controlled by the circadian clock. This mechanism is very similar to how daylength is measured for photoperiodic reactions. Second, we found that the circalunar clock determines the lunar period by counting circadian clock cycles, similar to a photoperiodic counter. Third, we substantiated that in *C. marinus* two developmental arrests, one induced by the circalunar clock and one induced by the photoperiodic counter, occur in the same developmental stage. Using a transcriptomics approach, we found that the circalunar developmental arrest in *C. marinus* may in fact be a mini-diapause. All these lines of evidence render the circalunar clock a prominent example of evolutionary tinkering. At the same time, our findings underline that the circalunar clock mechanism of marine insects is distinct from those of marine algae and annelids. This allows us to conclude that circalunar clocks evolved several times and are built along different functional principles in different branches of the tree of life. Therefore, they are an intriguing example for convergent evolution.



# Posters

## Chloramphenicol and gentamicin reduce resistance evolution to phage $\Phi$ X174 by suppressing a subset of *E. coli* C LPS mutants

Lavisha Parab , Jordan Romeyer Dherbey , Norma Rivera , Michael Schwarz , Jenna Gallie<sup>1</sup> , Frederic Bertels

Max-Planck Institute for Evolutionary Biology

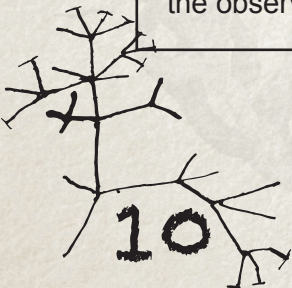
Bacteriophages infect Gram-negative bacteria by attaching to molecules present on the bacterial outer membrane, often lipopolysaccharides (LPS). Modification of LPS can lead to resistance to phage infection. In addition, LPS modifications can impact antibiotic susceptibility, allowing for phage-antibiotic synergism. The evolutionary mechanism(s) behind such synergistic interactions remain largely unclear. Here, we show that the presence of antibiotics can affect the evolution of resistance to phage infection, using phage  $\Phi$ X174 and *Escherichia coli* C. We use a collection of 34 *E. coli* C LPS strains, each of which is resistant to  $\Phi$ X174, and has either a “rough” or “deep rough” LPS phenotype. Growth of the bacterial strains with the deep rough phenotype is inhibited at low concentrations of chloramphenicol (and, to a much lesser degree, gentamicin). Treating *E. coli* C wildtype with  $\Phi$ X174 and chloramphenicol eliminates the emergence of mutants with the deep rough phenotype, and thereby slows the evolution of resistance to phage infection. At slightly lower chloramphenicol concentrations, phage resistance rates are similar to those observed at high concentrations; yet, we show that the diversity of possible mutants is much larger than at higher chloramphenicol concentrations. These data suggest that specific antibiotic concentrations can lead to synergistic phage-antibiotic interactions that disappear at higher antibiotic concentrations. Overall, we show that the change in survival of various  $\Phi$ X174-resistant *E. coli* C mutants in the presence of antibiotics can explain the observed phage-antibiotic synergism.

## Characterization of polymorphic inversions in locally adapted populations of *Clunio marinus*

Carolina M. Peralta & Tobias S. Kaiser

Max Planck Institute for Evolutionary Biology

Chromosomal inversions can play an important role in local adaptation, especially when local adaptation is being mediated by multiple and/or polygenic traits. By reducing recombination in heterozygotic state, inversions allow for the maintenance of co-adapted alleles. Even though there is a recent increase in studies focusing on inversions, the processes by which they arise, their role in maintaining multiple locally adaptive traits and how they are influenced by selection still requires further studies. In the marine midge *Clunio marinus*, locally adapted timing phenotypes can be observed in the intertidal zone of the European coast. As tidal regimes differ along the coastline, *Clunio* populations differ in daily and lunar emergence time, and these differences are genetically determined. Here, we explore the role of inversions in local adaptation in 15 *C. marinus* populations. Using SNP data, we detect nine large genomic regions in high linkage disequilibrium, and with long-read sequencing data we confirmed that at least five of these correspond to inversions. We characterized the inversion breakpoints and found that the breakpoint positions in the inverted haplotypes often co-occur with repeated regions. Interestingly, all the inversions are paracentric (i.e. they do not include the centromeres). After genotyping the inversions across all populations, we tested if inversion frequencies are correlated with emergence timing or geographical latitude. Our results shed light on the direct effects of inversions by gene disruption and indirect effects via recombination reduction in local adaptation of *C. marinus* populations.



# Posters

## Opposites attract: Innovation is maintained by disassortative mating and female choice

Alexandros Vezyrakis, Fragkiskos Darmis, Valeria Mazza, Anja Guenther

Max Planck Institute for Evolutionary Biology

Smarter, more innovative individuals are universally believed to be attractive, but then why are not all individuals in a population equally smart? Not all individuals in a population can innovate at the same rate or even innovate at all, yet it remains unclear how these differences are maintained. Here we combine observational and experimental approaches to show, for the first time, that female choice through disassortative mating can maintain differences in innovation. We examined how female house mice of different innovative skills choose their mates in semi-natural conditions, where we observed that individuals of opposite skills mate more often than expected by chance. In a follow-up two-choice controlled experiment, we detected the same pattern, providing direct experimental evidence for disassortative mating. Males face a trade-off between innovative skills and size, the latter being a predictor of competitive potential, and females of different skills prefer different males: innovative females prefer larger but non-innovative males while non-innovative females prefer innovative males regardless of size. Female preference and male trade-offs thus create the conditions for sexual selection to maintain variation in innovation, a pattern that is revealed only when concurrently investigating the same trait in both sexes.

## Patterns of genomic changes during adaptation of complex traits in small and large populations

Siva Subramanian Ayeraselvan, Claudia Ramirez-Lanzas, Neda Barghi

Max Planck Institute for Evolutionary Biology

Adaptation of complex traits involves genomic changes ranging from selective sweeps model, where individual loci rise to fixation, compared to polygenic model, characterized by subtle shifts across numerous loci both heavily influenced by numerous evolutionary pressures. This project examines allele frequency dynamics and haplotype block evolution during adaptation to a high-protein diet in small and large fruit fly populations. By analyzing significant shifts and structural changes, we aim to distinguish between selective sweeps and polygenic adaptation. These findings will enhance our understanding of how population size shapes genomic signatures of adaptation and the mechanisms driving the evolution of complex traits.

# Posters

## Growth inhibition between closely related strains of *Pseudomonas fluorescens*

Christine M. Bochynski , David W. Rogers, Paul B. Rainey

Max-Planck Institute for Evolutionary Biology

*Pseudomonas fluorescens* SBW25 is a well-studied model for evolutionary experiments investigating adaptation to abiotic challenges. Our goal is to extend these experiments to include biotic challenges. As a first step, we have characterized antagonistic interactions among 16 close relatives of SBW25. We found that interactions between strains were predominantly neutral, while some pairs exhibited strongly antagonistic effects mediated by diffusible toxins. SBW25 and its two closest relatives had clear growth-inhibiting phenotypes against nearly all other strains. A more distant relative demonstrated a varied growth-inhibiting phenotype towards targets, including a strongly antagonistic effect on SBW25. Resistance to this toxin in SBW25 was found to be associated with SNPs in the siderophore receptor gene *fpvB*. These antagonistic interactions have enormous potential to shape evolutionary dynamics, prompting the need to understand how these incompatibilities arise between close relatives. We hope to identify the genetic basis of both toxin production and immunity with the aim of understanding the evolution of both traits.

## More fathers under good or worse? The role of environmental quality in shaping polyandry

Fragkiskos Darmis

Max-Planck Institute for Evolutionary Biology

Historically, females have been described as choosy and eager to mate only to fertilize their ova. In contrast, males are predisposed by sexual selection to seek multiple matings because anisogamy, female pregnancy and limitations to access to females all create steeper sexual selection gradients for them. Consequently, multiple mating has been considered positively associated only with male fitness. For example, under a sexual conflict framework, polyandry is the byproduct of male coercion. However, male coercion might only partially explain the occurrence of polyandry and of byproducts of it. The last decades, polyandry has been found to be adaptive, taxonomically widespread and linked to increased reproductive success across human and non-human animals. With this study, using female house mice which are known to be promiscuous and have multiple fathers within the same litter, we show that the effect of polyandry are more nuanced than previously thought: while within environments promiscuity confers clear benefits, food-quality and availability might underlie the strength of such associations between mating and reproductive success. Specifically, in lower-quality environments, the effect of multiple male mates is greater. Our results clearly demonstrate that we have neglected an important mediator of the covariation between reproductive decisions and reproductive success, that is environmental quality.



# Posters

## Multilevel selection model for microbiomes

Amanda de Azevedo-Lopes , Arne Traulsen

Max-Planck Institute for Evolutionary Biology

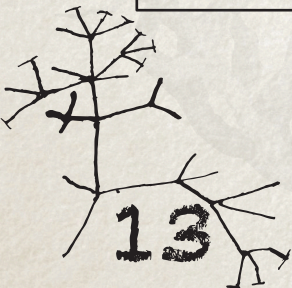
The study of multilevel selection within host-associated microbiomes has important implications for understanding the origin and evolution of these complex associations. To date, our understanding of the various levels influencing selection on microbial communities remains incomplete. The higher level of selection provided to the microbiome by the host has a substantial impact on the evolution of microbial lineages, favoring interactions beneficial on the higher level [1,2,3]. At the individual level, microbial lineages within each host are subject to selection favoring individuals with higher reproduction rates. Conversely, the health status and reproductive success of hosts may be enhanced depending on their microbiome, thereby increasing the chances of proliferation for the microbiome. The typical theoretical model contains two types of individuals: cooperators and defectors. Defectors tend to dominate over cooperators at the individual level, whereas groups composed entirely of cooperators have an advantage at the group level. This scenario illustrates how selection can operate in opposite directions across different levels - cooperation is beneficial at a higher level, but can be detrimental for an individual at a lower level. While previous research [1,2,3] has investigated the effects of higher-level selection, it has predominantly focused on the evolution of interactions among only two types. We expand this perspective by examining the potential role of multilevel selection in shaping the dynamics of host-microbiome interactions, particularly when considering a diverse population of microbial types. We seek to understand how multilevel selection influences the selection of interactions among various microbial types, whether it promotes higher microbial diversity within the population, and whether it increases the likelihood of microbial lineages evolving beneficial interactions with their host and other microbes. To address these questions, we structure a population of individuals into groups, where individuals interact through an evolutionary game that determines their fitness. Individuals immigrate from an environmental pool, reflecting an influx of microbial types. Within the groups, individuals are subject to birth and death as well as group division and extinction. Group sizes are constrained and when a group reaches a certain size, it either splits into two and another group is removed or a random individual from the group dies, such that the maximal group size is not exceeded. Through this process, multilevel selection is triggered by individual reproduction and constraints imposed by population structure. We show the impact on the interaction patterns emerging in such a system. [1] A. Traulsen and M. A. Nowak, "Evolution of cooperation by multilevel selection," *Proceedings of the National Academy of Sciences*, vol. 103, pp. 10952–10955, July 2006. [2] S. van Vliet and M. Doebeli, "The role of multilevel selection in host microbiome evolution," *Proceedings of the National Academy of Sciences*, vol. 116, pp. 20591–20597, Oct. 2019.

## Inferring Robust Species Trees in the Genomic Era

M. Rasit Durak , Sébastien Puechmaille, Celine Scornavacca, Frederic Delsuc, Julien Y. Dutheil

Max-Planck Institute for Evolutionary Biology

In evolutionary biology, inferring phylogenies has traditionally relied on single-locus data, but the genomic era has transformed this approach by enabling the integration of entire genomes. This shift to a multi-locus, phylogenomic framework brings new challenges, as different loci may carry complementary yet conflicting evolutionary signals, resulting in gene tree discordance. Such incongruence results from evolutionary events and mechanisms such as gene duplications, loss and transfer, as well as incomplete lineage sorting (ILS), which complicate the process of constructing a species tree that accurately reflects true evolutionary relationships and history. In this context, reconciliation methods have emerged as powerful tools for inferring the species tree from multiple gene trees, attempting to model biological incongruence directly. These methods harness the information in gene tree conflicts, providing a way to account for inherent evolutionary complexity. However, this modeling comes with additional computational costs and simplifications are needed, such as primarily focusing on a single source of conflict, like ILS. Despite their biological realism, reconciliation approaches leave open questions, such as how to assess the robustness of the resulting phylogeny. To address these challenges, we implemented a two-fold validation approach to evaluate the robustness of the inferred species tree, using Ascomycota, a diverse fungal phylum, as a benchmark. This approach combines direct topological measures with concordance metrics, allowing us to assess the robustness of the inferred tree. Using criteria based on branch support values, branch lengths, taxonomic groupings, and using randomization procedures, we tested whether observed patterns in the tree derive from biological signals or random variation. Indirect measures, such as gene concordance factors (gCF) and Robinson-Foulds distances, further strengthened the validation framework, capturing the impact of alignment length and other factors on tree congruence. Our comprehensive approach shows the importance of multi-angle validation in phylogenomics. By applying these checks, we gain confidence that the inferred Ascomycota species tree reflects genuine evolutionary relationships, showcasing the need for careful, rigorous assessment in the quest for accurate species phylogenies in the genomic era.



# Posters

## Does the neutral theory of molecular evolution apply to fungal pathogens?

Julien Y. Dutheil and Eva H. Stukenbrock

Max Planck Institute for Evolutionary Biology

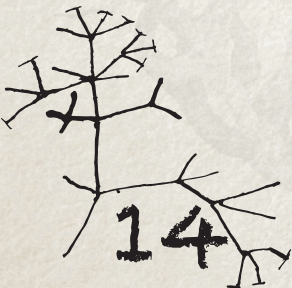
YES (Many fungal pathogens display a particular genome architecture, with genes directly involved in pathogenicity - the so-called effector genes - located in regions of the genome enriched in transposable elements (TEs). This observation led to the two-speed genome hypothesis, which stipulates that TEs trigger an increased variation in these genes that are preferential targets of adaptation, therefore being advantageous. We introduce a two-locus model with a genetic-distance modifier representing the accumulation of TEs between the two loci. We show that despite negative selection acting against TEs, they can invade when flanking genes are under strong positive selection - because linkage disequilibrium locally reduces the efficacy of purifying selection. We further extend the model to incorporate a possible effect of TEs on the mutation rate at the flanking loci. We show that such an effect generally acts against TEs, as the extra mutations would be, on average, deleterious. Our results suggest that TE accumulation is the consequence of rapid adaptation rather than its cause. Therefore, the two-speed genome pattern is better explained by a neutral hypothesis involving linked selection rather than an adaptationist view invoking a beneficial effect of TEs.)

## Can genetic promoters create mutational hotspots?

Andrew D. Farr, Christina Vasileiou, Peter A. Lind and Paul B. Rainey

Max-Planck Institute for Evolutionary Biology

Mutation rates can vary within genomes, changing in the position and occurrence of genetic variation that fuels evolution. Understanding the causes of this variation is important if we aim to make evolutionary biology a predictive science. We have recently taken advantage of a chance observation to show genetic regulation can influence mutation rates in a promoter of transcription. Experimental evolution with populations of *Pseudomonas fluorescens* revealed remarkable levels of parallel molecular evolution, with adaptive mutations caused by identical point mutations inside the *rpoS* promoter. A genetics approach has shown these mutations are driven by a mutational hotspot, with positive regulatory proteins required for a maximum mutation rate (~5700-fold above expectation). This poster presents resulting predictions and concepts: the conditions required for similar mutational hotspots in other genes and species, the ecological consequences of this hotspot, and the consequence of variation in gene expression on evolutionary processes.



# Posters

## The evolution of gene expression noise across yeast species

Fernanda Giersdorf, David Rogers, Julien Dutheil

Molecular Systems Evolution Group, Max-Planck Institute for Evolutionary Biology

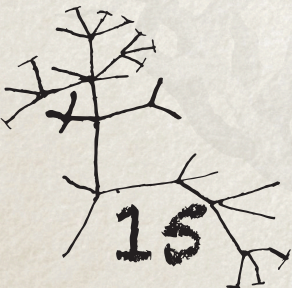
The development from genotype to phenotype through gene expression happens via inherently stochastic encounters and interactions of molecules. The evolution of regulatory mechanisms in gene transcription and translation, which are energy-costly, led to different levels of gene expression variation across genes within genomes and between species: Genes whose expression levels significantly impact the cell phenotype are likely to be finely regulated and maintained at an optimal expression level. On the other hand, a population of cells can benefit from adopting bet-hedging strategies in the expression of genes associated with environment sensing, allowing higher fluctuations in their expression levels from cell to cell. To understand the evolution of expression noise, we leverage phylogenetic comparative analysis of single-cell transcriptomes across 14 unicellular Saccharomycetes species. To our knowledge, these are the first genome-wide comparative analyses of transcriptional noise across species and the first single-cell transcriptional data available for these species. We show that expression noise is a generally rapidly evolving trait, and identified gene families with conserved high or low levels of transcriptional noise.

## The Role of a Biological Clock in Speciation

Alexander Jacobsen, Tobias S. Kaiser

Max-Planck Institute for Evolutionary Biology

Elucidating the mechanisms which precipitate speciation is central to our understanding of biodiversity and evolution. Here we investigate the potential role that biological clocks can play in the speciation process by exploring the ecological and molecular determinants of reproductive timing diversification in the marine midge *Clunio marinus*. *C. marinus* inhabits the intertidal zone of rocky European shores and is notable for timing its life history to the lunar cycle via an endogenous circalunar clock, such that whole populations will emerge and reproduce in synchrony on the extreme low tides surrounding full or new moon. Curiously, along the coast of Roscoff in France a population exists comprising two sympatric chronotypes, i.e. subpopulations that have diverged in their reproductive timing, one emerging only at full moon, the other one only just before new moon. This raises three questions: What was the source of divergent selection driving these chronotypes apart? Are differences in reproductive timing enough to stop gene flow and keep chronotypes genetically distinct? And what was the molecular basis of this shift in timing? By constructing an individual based model, we were able to show that the described reproductive ecology of *C. marinus*, when coupled with competition for space, provided the source of divergent selection. Next, through collecting mating couples in the field and controlled mating experiments in the laboratory, we found that while timing differences do pose a significant barrier to gene flow, further assortative mating and hybrid infertility strengthen this barrier and appear to be pleiotropic by-products of clock divergence. Lastly, through a genome-wide association study we discovered genes involved in timing differences between chronotypes and found that the ecdysis pathway and the core circadian clock were central to the divergence in reproductive timing. We conclude that the circalunar clock was the main driver of the incipient speciation for *C. marinus* chronotypes, highlighting the role that biological clocks can play in the speciation process.





# Posters

## Genetic tuning and evolutionary origins of the internal stop codon in prfB of *Pseudomonas fluorescens* SBW25

Sungbin Lim, Frederic Bertels and Jenna Gallie

<sup>1</sup>Max-Planck Institute for Evolutionary Biology

Translation termination is the last step in bacterial translation and involves the recognition of stop codons (UAA, UGA, UAG) by release factors (RFs). The efficiency of stop codons varies considerably, depending on the identity of the stop codon, genomic context, and physiological conditions. While most stop codons are fairly efficient, the prfB gene - encoding RF2, which recognizes UGA stop codons - carries an inefficient stop codon within its coding sequence. During translation, the recognition of the internal stop by RF2 (leading to severely truncated RF2) competes with a +1 ribosomal frameshift event (leading to full-length RF2). Since the balance of these two alternatives depends on RF2 concentration, the internal stop acts as an elegant autoregulation system. In this study, we investigate the evolutionary origins of the internal stop codon of the prfB gene and the subsequent tuning of termination efficiency. In particular: (i) can the efficiency of the prfB internal stop be manipulated, and (ii) what are the molecular and evolutionary consequences? So far, we have used genetic engineering to make changes to the efficiency of the prfB internal stop in the model bacterium *Pseudomonas fluorescens* SBW25. Through the evolution experiment, the engineered internal stop codon was compensated by two separate strategies, adjusting the reading frame upstream of the internal stop codon to bypass or mutate genes related to translational fidelity to increase the general frameshifting rate. The result might suggest a mechanism for the evolution and loss of RF2 autoregulation across the phylogeny. To summarize, our study experimentally investigates the evolution of RF2 autoregulation, gain and loss of autoregulation, and suggests hypothetical mechanisms for how the efficiency of an internal stop codon in prfB is tuned.

## How unstructured ecological differences drive population size fluctuations

Emil Mallmin, Arne Traulsen, Silvia De Monte

Max Planck Institute for Evolutionary Biology

Not only are ecological and evolutionary processes deeply entwined; the fundamental processes in community ecology - species selection, drift, dispersal, and diversification - are, conceptually, community-level counterparts to the fundamental population genetics processes - gene selection, drift, flow, and mutation. In both arenas, the relative importance of selection to neutral drift in explaining variation in frequencies (of species within the community, or alleles in the population) has been a source of much debate. The statistics of population size fluctuations (i.a. species abundance fluctuations) is of particular importance to this question. In ecology, empirical rates of compositional turnover in communities provide a decisive argument against purely neutral dynamics. In evolution, neutral molecular evolution remains controversial, because determining whether an allele is undergoing selection or not is obfuscated by several factors, including population size fluctuations. A null model for the population dynamics of a species embedded in an active ecological community is motivated. In my poster, I show how selective yet unstructured ecological processes - heterogeneous species interaction, or fluctuating environments - lead to boom-bust population dynamics of focal species within species-rich, competitive communities.



# Posters

## The Role of PRDM9 in Squamate Evolution and Speciation

Rahul Manshani , Nathalie Feiner , Linda Odenthal-Hesse

Max-Planck Institute for Evolutionary Biology

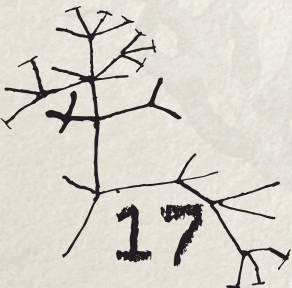
PRDM9, a pivotal regulator of meiotic recombination, determines recombination hotspots and facilitates the repair of double-strand breaks during meiosis. Despite its critical role, PRDM9 exhibits intriguing evolutionary patterns, including gene loss and functional redundancy, across the tree of life. Scaled reptiles (squamates) are particularly notable, with documented cases of PRDM9 loss in certain lizards and redundancy in some snakes. Our research explores the evolution of PRDM9 in squamates, focusing on the presence of pseudo- or complete genes and inter- and intra-genus variation in species from the genera *Podarcis* and *Anolis*. We aim to expand this analysis to other squamate families, including additional lizards and snakes. Given that PRDM9 is the only known speciation gene in vertebrates, we hypothesize that gene incompatibilities between the wild-type and nigriventris phenotypes of *Podarcis muralis* in the Italian hybrid zone may drive reproductive isolation. By characterizing interspecific variation in *Podarcis* and sequencing PRDM9 across pure and hybrid populations, we aim to clarify its role as a post-zygotic barrier and its potential involvement in speciation events within these populations.

## Investigating the dynamics of molecular evolution in microbial communities

Sandhya Lakshmi Narayanan

Max-Planck Institute for Evolutionary Biology

Molecular evolution allows the genetic study of living organisms, particularly in microbial populations where evolution can be observed in real time. While the dynamics of molecular evolution in individual microbial populations have been extensively studied—most notably in Richard Lenski's long-term *Escherichia coli* experiments—the impact of microbial communities in this context is less understood. In particular, the role of mobile genetic elements (MGEs) is understudied. MGEs can facilitate their own movement and inadvertently transfer ecologically significant genes between species, potentially influencing the fitness of recipient bacteria and the microbiome of host organisms. To gain clearer insight into the dynamics of molecular evolution in microbial communities and understand the importance of MGEs in this complex process, we will use the gut microbiome of the nematode *Caenorhabditis elegans*. By marking a single representative of the dominantly found genus *Ochrobactrum* with antibiotic resistance and fluorescent protein markers, we aim to observe the dynamics of molecular evolution within this bacterium with two treatments : in an isolated population and in the presence of an extracted microbiome from the nematode gut. Comparison of patterns of variation between the two treatments will allow the impact of a microbial community on the dynamics of molecular evolution of a single focal genotype to be determined.



# Posters

## Experimental evolution strategies to modulate bacteriophage life-history traits

Manuela Reuter , Michael Sieber , Octavio Reyes-Matte , Jordan Romeyer-Dherbey , Christopher Böhmker , Frederic Bertels , Javier Lopez-Garrido

Max-Planck Institute for Evolutionary Biology

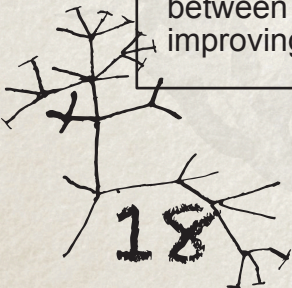
Bacteriophage's life-history traits, such as the rate of attachment to the host, the duration of the infection cycle, the number of progeny produced per infected cell, and the ability to persist in the environment, determine whether a phage infection is productive. Here, we show that subtle differences in transfer times during serial passaging can select for drastically different phage life-trait phenotypes. Long transfer times select for small plaque-forming phages that attach fast to host cells, produce large numbers of progeny per infected cell, and can persist in the environment for long periods of time. In contrast, short transfers select for large-plaque phages that attach slowly to host cells and decay rapidly in the environment. Small- and large-plaque formers differ by only a single point mutation in the capsid protein. Moreover, there is a very limited number of mutations that lead to the small plaque phenotype: we have repeatedly observed only three different mutations. In contrast, many different mutations turn a small-plaque mutant into a large-plaque former. The proximity of these very different phenotypes in genotypic space may be the result of frequent adaptation to different environments that select for life history traits associated with a specific plaque phenotype. Our results demonstrate that evolution experiments can be used to efficiently switch between these phenotypes, which may also be important for improving the efficacy of therapeutic phages.

## How homologous recombination shapes the genomes of naturally competent *Pseudomonas fluorescens*

David W Rogers , Ellen McConnell , Inga Schmidt , Kaumudi H Prabhakara , Paul B Rainey

Max-Planck Institute for Evolutionary Biology

*Pseudomonas fluorescens* SBW25 is a powerful model for the experimental study of evolution in the laboratory, but our understanding of the SBW25 genome and how it maps to phenotype has been limited by the availability of closely related comparisons. We have sequenced and assembled the genomes of 20 close relatives of SBW25 and found that these strains form a single recombining population. Comparing these genomes allows bioinformatic estimation of the influence of homologous recombination in generating variation between strains. To complement this analysis, we are developing protocols to promote natural competence in these strains, allowing us to directly quantify the effects of homologous recombination.



# Posters

## How do organisms adapt to fluctuating environments?

Hinrich Schulenburg

Max-Planck Institute for Evolutionary Biology, Kiel University

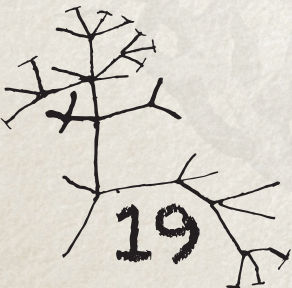
All organisms are subject to environmental fluctuations. Yet, to date, we lack a systematic understanding of how organisms adapt to such fluctuations, especially if irregular and unpredictable over time. Even though this topic has been addressed with the help of theoretical approaches, empirical and particularly experimental data is missing. Most previous experimental work has focused on set-ups, in which fluctuations were implemented by the regular alternation between two conditions. Thus, these experimental designs do not capture the situation in natural environments, where fluctuations are usually irregular (i.e., stochastic) and include more than two conditions. With this poster, I will use fluctuations in antimicrobial compounds as an example, which (i) can be observed in nature, (ii) shapes microbial communities in diverse contexts (e.g., agriculture, human medicine, natural environments) and (iii) is amenable to experimental analysis. Based on this example, I will discuss alternative strategies that can help organisms to cope with stochastic environmental fluctuations and options, how to test and further investigate these.

## A coarse-grained model of bacterial metabolism and the evolution of antibiotics

Michael Sieber

Max-Planck Institute for Evolutionary Biology

The anthropocentric view of antibiotics regards them as tools of bacterial competition that we very successfully use to fight bacterial infections. A more nuanced view takes into account that in natural bacterial populations antibiotics are often produced at low concentrations, which do not necessarily inhibit growth, but still have a wide range of effects on genetic and metabolic functions. Ribosome-acting antibiotics for example change the cellular composition of the proteome long before clinically relevant or resistance-inducing concentrations are reached. Here I present a coarse-grained model of bacterial metabolism to explore the idea that in at least some scenarios, antibiotics can re-structure a population's global proteome to better match environmental conditions. A case where this may be especially relevant is bacterial cross-feeding, where amino acids are produced and consumed by different sub-populations.



# Posters

## Evolution of interactions in yeast-bacteria communities in fluctuating environments

Kaumudi Prabhakara, Nina Wildenhayn, Ernesto Berrios-Caro, Paul Rainey

Max Planck Institute for Evolutionary Biology

Community level evolution is an important, but understudied aspect of evolution. Directed evolution of communities can not only increase our understanding of evolution on multiple scales, but also lead to the assembly of communities with desired functions. Here, we are interested in the co-evolution of communities in fluctuating environments. In particular, we ask how the interactions between the constituent species of a community evolve as the community is propagated cyclically through different environments. We cycled a community consisting of yeast and lactic acid bacteria (LAB) through two environments – one where the yeast grew well, but the LAB could not grow without yeast, and another where the LAB grew well, but not the yeast. After growth in the first environment, the communities with highest biomass were selected and propagated. After several rounds of propagation, we found that (1) on average, the biomass of communities in the first environment did not change, (2) on average, the biomass of communities in the second environment decreased and (3) new interactions, both positive and negative, had evolved between the yeast and LAB. To better understand our results, we use the competitive Lotka-Volterra equations to model this system, simulated via the Gillespie algorithm.

## Face to face: Transcriptomics and genomic regulation of facial prominences in mouse and chicken embryos

Stella Kyomen, Louk W. G. Seton, Elio Escamilla-Vega, Andrea P. Murillo-Rincón, Janina Fuss, Laura E. Cook, Axel Visel, Markéta Kaucká

Max Planck Institute for Evolutionary Biology - Kaucka Lab

The vertebrate face displays remarkable diversity in structure and function, shaped by intricate developmental processes. In amniotes, the frontonasal ectodermal zone (FEZ) is a key organizer of facial morphology, releasing morphogens that regulate the growth, polarity, and patterning of the underlying ectomesenchyme. Despite shared molecular pathways, species-specific differences in morphogen expression levels contribute to morphological variation. Here, we present a detailed single-cell analysis of the regulatory regions and gene expression patterns in the FEZ and underlying ectomesenchyme in mouse and chicken embryos. Our data reveal how distinct gene regulatory landscapes are calibrated to drive differential facial morphogenesis between species. By linking cis-regulatory elements to facial morphologies and integrating results with GWAS data, we explore natural variations in facial shape and congenital malformations. These findings provide a comprehensive framework for evolutionary studies and illuminate the regulatory mechanisms underlying facial diversity in amniotes, offering insights into how genetic variants contribute to facial traits and developmental anomalies.



# Posters

## Cellular heterogeneity and developmental patterning of facial mesenchyme

Louk Seton, Andrea P. Murillo-Rincón, Elio Escamilla Vega, Markéta Kaucká

Max Planck Institute for Evolutionary Biology

Vertebrates exhibit an extraordinary diversity of facial shapes, allowing them to develop diverse strategies and specialized functions. The spectrum of facial geometries is generated mainly during embryonic development, in an intricate morphogenetic process defined by dynamic cell behavior and increasing cellular complexity. Long-standing attempts to comprehend the evolutionary divergence of craniofacial development are hindered due to the lack of detailed knowledge of cellular processes and their molecular underpinnings. Here, using single-cell transcriptomics, in situ Hybridization Chain Reaction, and cellular communication prediction, we reconstruct molecular events and their coordination during early embryonic face formation in a mouse model. The establishment of the cellular and molecular landmarks during face formation and shape acquisition will aid in the identification of cell populations linked to natural intra-species face variability and pinpointing molecular players involved in congenital facial syndromes.

## Meiosis reinvented – stage-specific PRDM9-independent gene expression patterns during spermatogenesis

Jasmin Cichy, Orion Pirku, Martin Speckmann, Gabriela Michel, Nicole Thomsen, Sven Künzel, Axel Wehrend, Alan Derman, Kristian Ullrich and Linda Odenthal-Hesse

Max Planck Institute for Evolutionary Biology

In order to produce healthy offspring, both female and male of any sexually reproducing species must have healthy gametes with correctly segregated chromosomes – a process that in almost all metazoans is contingent on PRDM9. As the leading regulatory protein during meiosis I, PRDM9 marks specific locations throughout the genome and recruits a machinery of proteins that induces DNA strand breakage in these sites. Repair of these breaks can lead to genetic exchange between homologous chromosomes and hence genetic variation in the offspring, facilitating evolution. Upon loss or mutation of PRDM9, meiosis will be arrested in early stages and the germcells won't continue in their development, often leading to infertility. However, very few animal groups evolved to lose PRDM9 function without these cytotoxic effects, including birds and canids. Fascinatingly, these animals remain fertile while exhibiting the same genetic phenotype as sterile PRDM9-knockout mutant mice. To start understanding how PRDM9 can be essential for germcell development in most species but evolutionarily lost in others, we here focus on PRDM9s' teamplayers: By immunostaining proteins involved with meiotic recombination, we compared gene expression patterns of PRDM9-dependent mice with PRDM9-independent birds and dogs throughout individual meiotic stages. Unraveling genetic (in)dependencies, interactions and effects upon loss of function of PRDM9 provides first insights into the pathways that compensate for PRDM9s' function in birds and canids.



# Posters

## Short- and long-term strategies for the evolution of nascent multicellular life cycles

Gisela T. Rodriguez-Sanchez, Joanna Summers, Paul B. Rainey

Max Planck Institute for Evolutionary Biology

The transition from single cells to multicellularity requires acquisition of Darwinian properties at the new-higher level of organization. This can happen through the evolution of a developmentally regulated life cycle, which has a collective and a propagules phase. Our experiment, which imposes group selection to populations of the bacterium *Pseudomonas fluorescens* SBW25 via a life cycle, alternating between mat formation (collective) and swimming behaviour (propagules), resulted in the evolution of lineages with different developmental programmes that regulate the transition through the cycle in 5 generations. We were interested in exploring whether the mutations history would influence the possibilities of acquiring further adaptation in the longer term. Thus, we allowed two focal lineages to continue evolving for further nine generations. We found that mutation history matters. Although the focal strategies seemed to be equally successful against the ancestor in the short-term, over the longer term, they differed in their extinction frequency, resulting in different strengths of between-group selection. Consequently, lineages also differed in accumulation of secondary mutations as well as the likelihood of fixation of adaptive mutations. Improvement at the lower-level was observed for both lineages, but fixation of these mutants in the population was constraint by spreading opportunities. Our results show that when lineages are the focus of selection, different forces can drive evolution. Population dynamics, as in the lower-level individual that form them, can be observed. The strength of selection as a consequence of the degree of competition between lineages will determine the rate of adaptation and drift can lead to fixation of adaptive and neutral mutations. Among the multiple strategies that can evolve, only those with both short- and long-term positive effects are likely to allow future evolutionary endogenization of externally imposed Darwinian properties.

## Neural crest cell gene expression in embryonic space and time. A mechanism for trait co-evolution?

Robin Pranter, Cedric Patthey and Nathalie Feiner.

Lund University

Neural crest cells are multipotent embryonic cells that contribute to a wide variety of vertebrate traits. Cell types derived from neural crest cells include osteocytes and chondrocytes that shape facial appearance, chromatophores in the skin giving vertebrates their colors and endocrine cells such as the chromaffin cells of the adrenal gland contributing to aggressive behaviors. As such, neural crest cells have been hypothesized to developmentally link seemingly disparate traits and make them evolve together. To test this hypothesis, we study a newly evolved complex phenotypic syndrome of neural crest cell-derived traits in the common wall lizard, consisting of exaggerated head size, coloration and aggressive behaviour. Here we present results from single cell transcriptomics and imaging to describe how neural crest cells are specified, migrate, and differentiate in the common wall lizard. The results reveal that neural crest cell development is mostly conserved with respect to other investigated vertebrates, however, we do not observe the second postotic stream reported in chameleon. This is a crucial first step towards our goal to test whether neural crest cells cause vertebrate traits to evolve in concert, giving direction to phenotypic evolution.

# Posters

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

Inês Borges, Niki Vontzou, Erik Enbody, Carl-Johan Rubin, Brian Davis, Leif Andersson, Valentina Peona, Francisco J. Ruiz-Ruano, Alexander Suh

Leibniz Institute for the Analysis of Biodiversity Change

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 DNA-rich 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.

## Evolution of bacterial mixed strain populations: A story of microbial interactions, standing genetic variation and antibiotic selection

Aditi Batra, Leif Tueffers, Kira Haas, Ernesto Berrios Caro, Tabea Loeblein, Joao Botelho, Gabija Sakalyte, Hildegard Uecker, Daniel Unterwiesing and Hinrich Schulenburg<sup>1</sup>

Wageningen University and Research, Netherlands

Many infections are polymicrobial in nature. In such infections, ecological aspects such as microbial interactions, horizontal gene transfer and standing genetic variation can act as drivers of antimicrobial resistance. In this study, we wanted to investigate the contribution of these factors to antibiotic resistance evolution in a mixed strain population of the opportunistic pathogen *Pseudomonas aeruginosa*. We found that microbial interactions and standing genetic variation significantly contributed to determining the population composition at the end of evolution under antibiotic therapy. Microbial interactions were further found to enable survival of the bacterial populations under strong antibiotic selection. While horizontal gene transfer was observed, it was not associated with antibiotic resistance. We conclude that ecological factors such as microbial interactions and standing genetic variation can positively influence the evolution of a mixed strain population of bacteria under stressors such as antibiotics. Our findings further the understanding of microbial interactions and their effect on evolution. They also highlight the necessity of considering ecological aspects of infecting populations during antibacterial therapy.



# Posters

## Analyzing the morphological and functional evolution of insect wings using a theoretical morphospace approach

Yuming Liu, Pablo Milla Carmona, Emily J. Rayfield & Philip C.J Donoghue

University of Bristol

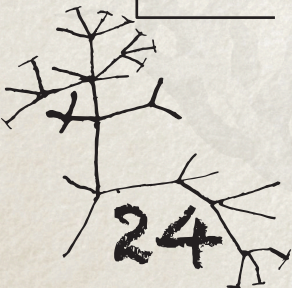
Insects are the earliest flying animals and, ultimately, the most diverse animal lineage. Aerodynamic performance is presumed to impose intense selection pressure on wing morphology and therefore many studies assume that insect wing morphology has also evolved to be optimal for flight. To test this hypothesis, we using a theoretical morphospace approach to characterise morphological and variation of wing shape, and accordingly constructed optimality landscapes to explore how function impact the evolutionary trajectories of insect wing morphology. With sampling over 4,000 species of insects to derive a theoretical morphospace that expanded beyond realized variation, from which theoretical wing shapes could be interpolated and analysed functionally. We tested the functional performance of theoretical wings through blade element analysis and finite element analysis to estimate aerodynamic force and breakage resistance, and then used a Pareto ranking approach to identify the optimal wing shape for the trade-off between these metrics. Additionally, we conducted a series of macroevolutionary analyses to explore the relationships between phylogeny, wing morphology, and functional optimality. Our results show that sampled wings perform highly for breakage resistance and moderately for aerodynamic performance, and moderately for their trade-off. This observation implies that natural selection has not led to the evolution of functionally optimal wing morphologies but, rather, toward local adaptive solutions that are functionally adequate.

## Are r and K genetically correlated?

Leonor R Rodrigues, André Mira, Inês Fragata, Claus Rueffler, Sara Magalhães

Centre for Ecology, Evolution and Environmental Changes, Faculty of Sciences, University of Lisbon & Wissenschaftskolleg zu Berlin

Per-capita growth rate at low population density and competitive ability are often assumed to trade off with each other. This is known as the r vs K trade-off. However, r and K could be positively correlated if individuals differ mainly in how efficiently they exploit resources. Here, we aim to address this issue by measuring growth and competition traits at the individual and population levels in 19 isogenic lines of the ectoparasitic spider-mite, *Tetranychus urticae* a major crop pest. Specifically, we measured 1) the intrinsic growth rate as the number of viable females produced from a single female in the absence of competition; 2) resource use as the quantity of resource consumed by a single individual, estimated as the amount of damage caused to a piece of leaf; 3) the equilibrium population size given a certain amount of resources corresponding to the carrying capacity; and 4) the degree of intraspecific competition, as the interference caused by other individuals on resource consumption. We found that the intrinsic growth rate was positively correlated to K, suggesting that some genotypes exploit the environment better than others. However, at the individual level, resource use correlated positively with interference, indicating that genotypes that require more resources are more affected by intraspecific competition. In conclusion, we do not find empirical support for a trade-off between r and K, but instead for a trade-off between resource use and the sensitivity to competition.



# Posters

## Steady-State vs Dynamics: Insights into the Evolution of Gene Regulatory Networks

Greshnova A., Iglar C., Lagator M., Guet C.

Yale University

The evolution of gene regulatory networks (GRNs) is often studied through changes in steady-state expression levels caused by mutations in regulatory regions. However, the dynamic properties of gene regulation, such as the time required to reach steady-state and the evolution of temporal behaviours, remain poorly characterised. In this study, we empirically investigated how mutations in regulatory regions affect both the steady-state and dynamic properties of GRNs and their evolution. We used a canonical system to study gene expression - the genetic switch of lambdaoid phages - and created a synthetic GRN to characterise the effect of mutations in vivo. Further, we quantified the evolutionary potential of each trait as summarized by two key metrics: robustness and evolvability. Our results show that many mutants with robust steady-state expression exhibit significant variation in dynamic behaviour, shedding light on how these properties diverge during evolution. This nuanced characterisation of GRNs highlights the critical interplay between steady-state and dynamic properties, providing new insights into how evolution shapes phenotypic diversity and determines adaptive trajectories.

## A key innovation in the evolution of Lepidoptera

David Heckel

Max Planck Institute for Chemical Ecology

In modern lineages of the Lepidoptera, larvae consume green plants as their main food. To avoid phototoxicity 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.



# Posters

## Skull bones of contrasting developmental origins influence both micro- and macroevolution in lacertid lizards

Quentin Horta-Lacueva , Juliane Vehof, Morris Flecks, Tobias Uller, Nathalie Feiner

Lund University

While dating back to the Darwinian revolution, the importance of microevolutionary processes in macroevolution are yet unresolved. In the vertebrate skull, a bridge between micro- and macroevolution may reside in long-lasting developmental biases from bones with specific variational properties respective to their contrasting embryonic origin (i.e., neural crest vs. the mesodermal tissue). We tested this hypothesis by studying skull shape in the highly diverse family of Lacertid lizards, using X-ray microtomography-based (CT) 3D morphometric analyses. At the microevolutionary scale, we quantified variation in skull shape across populations of European wall lizards (*Podarcis muralis*) known to exhibit extreme morphological variations. At the macroevolutionary scale, we compare skull shape across more than 150 lacertid species. We quantified the contribution of the main skull bones to patterns of variation with regards to their neural crest or mesoderm origin. Family-level disparity patterns concordant with the variability observed in *P. muralis* would reveal if and how the neural crest biology underlies the possible integration of micro- and macroevolutionary processes.

## When are mutations that increase phenotypic noise beneficial?

Jun Ishigohoka, Luisa F. Pallares

Friedrich Miescher Laboratory of the Max Planck Society

There are opposing views on the evolutionary impact of phenotypic noise. On the one hand phenotypic noise can be beneficial because it allows organisms to explore a wider phenotypic space to adapt to new environments. On the other hand, it can be disadvantageous due to reduced robustness. For both claims, it is essential that variation in phenotypic noise is heritable for it to evolve by natural selection. Based on the infinitesimal assumption i.e. no major-effect loci, theoretical quantitative genetics predicts that heritable variation in phenotypic noise impacts the response to selection, which in turn affects the heritability of noise. However, genome mapping approaches have identified loci associated with phenotypic noise, indicating major loci do exist. Given such genetic architecture with major-effect loci, population genetic theory of noise-affecting mutations is needed. Specifically, little is known about how major-effect loci for phenotypic noise affect organisms' response to selection on traits. Using computer simulations, we discuss how mutations with major effects on phenotypic noise, in addition to the trait mean, respond to directional and stabilising selection. We also present the design of evolution experiments using the fruit fly *Drosophila melanogaster* to validate the simulation-based predictions. Our results are some of the first attempts to comprehensively understand in which population genetics parameter space robustness-regulating loci can evolve.



# Posters

## Unraveling the genomic underpinnings of parallel evolution in Mediterranean wall lizards

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Shared phenotypes among distantly related species provide compelling evidence of parallel evolution in response to common ecological pressures or developmental biases. Nevertheless, the genetic mechanisms underlying parallel evolution remain poorly known. Here, we perform a comparative phenomic and genomic study of Mediterranean wall lizards (genus *Podarcis*). We show that several *Podarcis* species independently evolved a similar suite of consistently correlated characters, including coloration, morphology, and behavior. In each species, this 'syndrome' is present in only some populations, which enabled us to investigate the genomic basis of the repeated evolution of complex phenotypes. To this end, we employed whole-genome re-sequencing and genome scans to identify and compare loci associated with the syndrome across species, finding both shared and unique outlier loci. This combined approach provides insights into the factors underpinning repeated evolutionary outcomes – a pervasive feature of life on Earth.

## Repeatability of genetic evolution in *Caenorhabditis elegans*

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Repeated adaptation to similar, but not identical, environments is common, including in bacterial resistance evolution and the emergence of new diseases. Nevertheless, little is known about how repeatable and ultimately predictable evolutionary processes are in general. In this study, we performed a large scale evolution experiment in which *Caenorhabditis elegans* was exposed to the novel dietary bacterium *Bacillus megaterium* and evolved over 15 weeks (~ 20 generations). Experiments were carried out in parallel at six research institutes under different conditions and whole genome sequencing (WGS) data of the populations was obtained after week 1 and 15. Additionally, the nematode fitness was measured at both timepoints. By analysing the genomic evolution of the nematodes, we identify candidate genes that potentially drive adaptation and ask whether the same or different genes underlie repeated adaptation. Making use of the many microbial sequences in the WGS data, we identify the microbiome associated with the *C. elegans* populations across different research institutes and conditions. Interestingly, we find distinct microbial profiles and several shared but also distinct genetic changes. Combining the worm and microbial genomic data allows us to investigate adaptation of *C. elegans* and find factors that influence its repeatability.



# Posters

## Conservation genomics of Anthophora bees

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Solitary bees are vital components of the ecosystem, but population declines have been reported for many species. *Anthophora* is a solitary bee genus containing ~400 species. Three species from this genus - *Anthophora quadrimaculata*, *Anthophora retusa*, and *Anthophora plagiata* - are all present widely in Europe, but significant regressions have been reported in parts of their range. In Sweden, both *A. plagiata* and *A. retusa* have experienced declines in recent decades to the point of local extinction, but substantial recovery has been reported in the case of *A. retusa*. We generated genome assemblies of these species using long reads, resulting in assemblies of 325 - 450 Mbp with contig N50 > 12 Mbp. All three genomes contain a high proportion of highly repetitive heterochromatin in which variant calling is highly error-prone. Population resequencing of 140 samples from throughout Sweden revealed that *A. plagiata* has extremely low genetic variation (heterozygosity = 0.01%) which is lower than reported for other highly threatened insect species. Surprisingly, however, subpopulations of this species are not genetically distinguishable despite geographical isolation. *A. retusa* does not exhibit reduced genetic variation despite population subdivision, indicating two recent independent recoveries from a bottleneck. Using MSMC, we find evidence for gradual long-term population declines for all species since the end of the Pleistocene and evidence that effective population size in *A. plagiata* has been historically low. These results are concordant with those from other insect species indicating that many threatened species have experienced population declines that predate the modern era.

## Microbiome-Mediated Host Adaptation: A Niche Construction Approach

Bob Week, Hinrich Schulenburg

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Host-associated microbiomes play a crucial role in adaptive evolution, often by mediating the host's response to environmental pressures. Niche construction theory (NCT) provides a valuable lens for understanding how hosts, in partnership with their microbiomes, actively modify their environment to enhance fitness and alter evolutionary processes. We present four distinct ways to apply NCT to understand different aspects of microbiome-mediated host adaptation, and illustrate each perspective with an empirical example. These perspectives are foundational for the development of theoretical frameworks to advance our understanding of host-microbiome systems with potential applications to conservation, agriculture, and human health.

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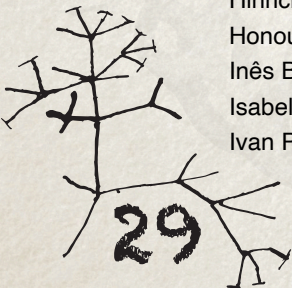




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