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Winter moth (*Operophtera brumata*) adaptation to climate change:

van Dis, Natalie Elisabeth; van der Zee, Maurijn; Hut, R.A.; Wertheim, Bregje; Visser, Marcel

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Abstract Book

Second Conference of the Netherlands Society for Evolutionary Biology

Tuesday, April 16th 2019 , *Akoesticum*, Ede, The Netherlands



Sponsors



BRILL

Organizing committee

Eveline Verhulst, Martijn Egas, Katja Peijnenburg, Marian Bemer, Bertus Beaumont, Sijmen Schoustra

Program

- 09:00-09:25 **Arrival** with coffee/tea
- 09:25-09:30 **Opening** by Marcel Visser (NLSEB Chairperson)
- 09:30-10:00 **Gusztai Eiben** (Vrije Universiteit Amsterdam)
“Evolution in wetware, software, and hardware: what can we learn?”
- 10:00-10:30 **Frietson Galis** (Naturalis Biodiversity Center)
“Developmental constraints and body plan evolution in animals”
- 10:30-10:45 **poster pitches**
- 10:45-11:15 **Coffee/tea**
- 11:15-12:15 **Parallel sessions** of contributed talks
Session I: Auditorium
Session II: Tuinzaal
- 12:15-13:15 **Lunch with network opportunities**
- 13:15-14:30 **Poster session**
- 14:30-15:00 **Geert Kops** (Hubrecht Institute-KNAW)
“Weird divisions: Evolutionary dynamics of eukaryotic kinetochores”
- 15:00-16:00 **Parallel sessions** of contributed talks
Session III: Auditorium
Session IV: Tuinzaal
- 16:00-16:15 **Tea/coffee**
- 16:15-16:30 **Poster pitches**
- 16:30-17:00 **General assembly** of NLSEB members
- 17:00-17:20 **Musical intermezzo: “The Happy Face Spiders”**
- 17:20-18:30 **Poster session** with drinks
- 18:30-18:35 Announcement **poster prize** winners
- 18:35-20:00 **Dinner with optional round-table discussions**

Sponsoring

Baseclear: Poster prize

Brill: Drinks

NWO: Meeting

Program parallel sessions

Parallel session 1

- 11:15-11:30** **Barbara Helm** (GELIFES), Evidence for evolutionary response in the advancing phenology of a migratory bird.
- 11:30-11:45** **Koen Verhoeven** (NIOO-KNAW, Terrestrial Ecology). Epigenetic inheritance of environmental effects in asexually reproducing plants.
- 11:45-12:00** **Jia Zheng** (GELIFES). Parental care patterns are associated with mating opportunities in the Chinese penduline tit *Remiz consobrinus*.
- 12:00-12:15** **Shraddha Shitut** (Microbial Biotechnology and Health (IBL), Supramolecular Chemistry (LIC)), Evolution of chromosomal coexistence

Parallel session 2

- 11:15-11:30** **Leo Beukeboom** (GELIFES). Molecular evolution of insect sex determination.
- 11:30-11:45** **Cas Retel** (Fish Ecology and Evolution, CH), The feedback between selection and demography shapes coevolutionary genomic change.
- 11:45-12:00** **Maurijn van der Zee** (Leiden, Biology). Eco-evo-devo of insect embryology.
- 12:00-12:15** **Tiziana Paola Gobbin** (GELIFES). Parasites as potential drivers of speciation in old and recent cichlid fish lineages.

Parallel session 3

- 15:00-15:15** **Frederic Lens** (Naturalis), Towards a better understanding why plants became woody during evolutionary history.
- 15:15-15:30** **Casper van der Kooi** (GELIFES), Tuning of flower colours to the visual systems of pollinators
- 15:30-15:45** **Katja Peijnenburg** (Naturalis/UvA, Marine Biodiversity). Early Cretaceous origin of pteropods suggests their resilience to ocean acidification.
- 15:45-16:00** **Sabrina Simon** (WUR Biosystematics). Evolutionary history of Polyneoptera and its implication for the evolution of winged insects.

Parallel session 4

- 15:00-15:15** **Duur Aanen** (WUR Genetics), Mutation-rate plasticity and the germline of unicellular organisms.
- 15:15-15:30** **Hilje Doekes** (UU, Theoretical Biology& Bioinformatics) What selection pressures shape the evolution of cell-density dependent toxin production in bacteria? Insights from a computational model.
- 15:30-15:45** **Enzo Kingma** (TUDelft, Bionanoscience), Evolution of Pleiotropic Networks Can lead to Environmentally Robust Phenotypes.
- 15:45-16:00** **Arjan de Visser** (WUR Genetics), Population size and the repeatability of antibiotic resistance pathways.

Abstracts Plenary Lectures

Evolution in wetware, software, and hardware: what can we learn?

Gusztai Eiben

Vrije Universiteit Amsterdam

In this talk I explain the basics of artificial evolution in software (Evolutionary Computing) and hardware (the Evolution of Things). I argue that such systems are interesting for engineering as well as for fundamental scientific studies. For instance, systems of self-reproducing robots can be used to investigate macro-mechanisms of evolution, the simultaneous evolution of bodies and brains, or the emergence of embodied intelligence. Specifically, I outline the concept of EvoSphere, summarize the “Robot Baby Project”, our first proof-of-concept implementation, and review on-going research projects. To conclude I discuss challenges and opportunities for joint research in the intersection of biology, Artificial Life, Artificial Intelligence, and robotics.

Developmental constraints and body plan evolution in animals

Frietsen Galis

Naturalis Biodiversity Center

Examples of well-documented hard developmental constraints on the evolution of adaptive phenotypes are rare in the literature. In all cases the constraints appear to be caused by complex and precisely controlled global interactivity of development that when disturbed has serious consequences. Mutations that affect such global interactivity almost inevitably have many harmful pleiotropic effects, and thus will be strongly selected against, leading to long-term evolutionary conservation. I will discuss two outstanding examples. The strongest and most universal of the constraints is the incompatibility of ciliated and differentiated animal cells to undergo mitosis. One consequence of this constraint is the early determination of almost all organ primordia, when development is still highly interactive (phylotypic stage). This leads to the second example, the constraint against changes of phylotypic stages in vertebrates and other higher taxa of animals. Both have major consequences for the evolution of body plans.

Weird divisions: Evolutionary dynamics of eukaryotic kinetochores

Jolien van Hooff, Eelco Tromer, Berend Snel, Geert JPL Kops

Onco Institute, Hubrecht Institute

The tremendous diversity in eukaryotic life forms can ultimately be traced back to evolutionary modifications at the level of cellular processes. A major challenge in biology is to understand how such modifications create diversity while maintaining core cellular functions. I will present our efforts on understanding diversity and evolution of an essential molecular machine, the kinetochore. Kinetochores are large protein assemblies that links centromeres to microtubules, and that emit molecular signals in order to safeguard the equal distribution of duplicated chromosomes over daughter cells. Although kinetochores are essential components of cell division, our phylogenetic analyses indicate extensive diversity in kinetochore composition across eukaryotic lineages. We use these analyses to 1) guide experiments on composition and function of divergent kinetochores; 2) trace the origins and evolution of kinetochore components; 3) mine orthologous families for co-evolving networks and functional protein features that may explain some of the observed diversity.

Abstracts Contributed Talks

(alphabetical order)

Mutation-rate plasticity and the germline of unicellular organisms

Duur Aanen and Fons Debets

Wageningen University

The mutation rate is a fundamental factor in evolutionary genetics. Recently, mutation rates were found to be strongly reduced at high density in a wide range of unicellular organisms, prokaryotic and eukaryotic. Independently, cell division was found to become more asymmetrical at increasing density in diverse organisms; some 'mother' cells continue dividing, while their 'offspring' cells do not divide further. Here, we investigate how this increased asymmetry in cell division at high density can be reconciled with reduced mutation-rate estimates. We calculated the expected number of mutant cells due to replication errors under various modes of segregation of template-DNA strands and copy-DNA strands, both under symmetrical (exponential) and asymmetrical (linear) growth. We show that the observed reduction in the mutation rate at high density can be explained if mother cells preferentially retain the template-DNA strands, since new mutations are then confined to non-dividing daughter cells thus reducing the spread of mutant cells. Any other inheritance mode results in an increase in the number of mutant cells at higher density. The proposed hypothesis that patterns of DNA-strand segregation are density dependent fundamentally challenges our current understanding of mutation rate estimates and extends the distinction between germline and soma to unicellular organisms.

Molecular evolution of insect sex determination

L.W. Beukeboom

University of Groningen

Sex determination is a fundamental developmental process, but the underlying genetic mechanisms are remarkably diverse. Sex determination in insects occurs by a cascade of hierarchically organized genes, consisting of a primary signal, transformer (*tra*) and doublesex (*dsx*). The cascade evolves from the bottom upwards with a large diversity and turnover of primary signals on top. Changes in sex determination pathways can occur via gene duplication, changes in gene function, shifts in gene hierarchies, and alterations of gene regulation. These processes lie at the basis of shifts between sex determination systems, such as male heterogamety, female heterogamety, monogeny, and haplodiploidy. The housefly, *Musca domestica*, is a good model species to study the evolution of sex determination mechanisms as it harbours different sex determination mechanisms, which can be used to unveil the structural genomic changes and gene regulation alterations involved. Such research will be instrumental for understanding the evolutionary dynamics of insect sex determination, but also has applied value, e.g. for improving insect mass rearing and pest control.

What selection pressures shape the evolution of cell-density dependent toxin production in bacteria? Insights from a computational model

Hilje Doekes, Rob de Boer and Rutger Hermesen

Utrecht University

Bacteria live in diverse microbial communities, in which they compete with neighbouring microbes for nutrients and space. In this competitive environment, almost all known bacterial strains produce toxins that impair or kill other bacteria. This chemical warfare is an example of spiteful social behaviour: bacteria invest resources to harm others. Many toxins are produced only if the local density of bacteria is high. To achieve this, bacteria respond to cell-density cues, which can be signalling molecules or other indicators of the presence of other cells. It is unclear which selection pressures shaped the evolution of such density-based regulation of toxin production. Here, we use computational modelling to study the evolution of density-based regulation of toxin production in spatially structured bacterial populations. We show that such regulation can arise under various growth conditions, and identify selection pressures driving its evolution. In particular, we find that if bacteria regularly colonise new habitats, density-based regulation allows them to express a fast-growing, non-toxic phenotype when expanding into uncolonised territory, and a slower-growing, toxin-producing phenotype when competing with other strains. Colonies of regulating cells show a typical structure with rapidly dividing, sensitive cells at their expanding edges, and slower dividing, toxin-producing cells in their interior and at the interface with other colonies.

Parasites as potential drivers of speciation in old and recent cichlid fish lineages

Tiziana Gobbin

University of Groningen

Parasites may have strong eco-evolutionary interactions with their hosts. Consequently, they may initiate or promote host diversification. The radiation of cichlid fish in Lake Victoria provides a good model to study the role of parasites in the early stages of speciation. We analysed the macroparasite fauna of 17 young cichlid species from a recent radiation, and 2 older species from 2 non-radiating lineages. Host species had different parasite infection profiles, which were only partially explained by ecological factors (diet, water depth). This may indicate that differences in infection are not simply the result of differences in exposure, but that hosts evolved species-specific resistance, consistent with parasite-mediated divergent selection. Infection was similar between sampling years, indicating that parasite-mediated selection is stable through time. The monogenean gill parasite *Cichlidogyrus*, is considered a good candidate for driving parasite-mediated speciation, because it is host species-specific and has radiated elsewhere in Africa. We morphologically identified 8 *Cichlidogyrus* species, but found no evidence that *Cichlidogyrus*-mediated selection contributes to the early stages of speciation. Instead, differences in infection seem to accumulate after speciation. To conclude, we find significant interspecific variation in parasite infection profiles. However, the association between species divergence and parasite infection depends on the parasite taxon considered.

Evidence for evolutionary response in the advancing phenology of a migratory bird

Barbara Helm, Benjamin Van Doren, Dieter Hoffmann and Ute Hoffmann
University of Groningen

Migratory birds may not be sufficiently advancing the timing of migration and reproduction to compensate for earlier springs, leading to population declines. Responses to early springs may be constrained by the inherited clock-based timing programmes that regulate long-distance migrations, making evolutionary adjustment the only viable mechanism for keeping pace with shifting phenology. Therefore, it is imperative to understand how rapidly migrants' circannual timing programmes can evolve. We replicated a laboratory investigation after 21 years of global warming to investigate whether annual-cycle timing had advanced in the pied flycatcher (*Ficedula hypoleuca*), a model for studying constrained ecological responses to climate change. We show that the phase of the flycatcher clock controlling spring moult, migration, and reproductive timing advanced by 6-12 days, whereas no such changes occurred in autumn and winter. A nearby wild population mirrored these changes, advancing egg-laying by 11 days. Furthermore, the time window during which wild flycatcher reproductive timing was most sensitive to ambient temperature advanced by three weeks. These results support a role of an evolutionary response in modifying spring phenology and suggest that the inherited timing programmes of long-distance migratory birds may be more labile than previously thought, leaving some scope for evolutionary rescue in a changing climate.

Evolution of Pleiotropic Networks Can Lead to Environmentally Robust Phenotypes

Enzo Kingma, Leila Iñigo de la Cruz, Eveline Diepenveen, Liedewij Laan
TU Delft

How biochemical networks evolve to increase fitness is not only determined by network topology, but also by environmental conditions. We investigate how these environmental factors interact with network topology during experimental evolution of a *bem-nrp1* mutant of *S. cerevisiae*, which is defective in its ability to polarize, in two conditions: (1) the relatively constant environment of a continuous culture and (2) the fluctuating environment of a batch culture. We show that the fitness cost of deleting Bem3 and Nrp1 is governed by epistasis and pleiotropic effects. In a constant environment, parallel evolution reveals that both mutations that improve or deteriorate the fitness of a trait that is not under selection can settle in a population by being connected to another trait that is under selection. In a fluctuating environment, simultaneous selection on both traits causes both of them to improve, resulting in the emergence of phenotypes that are similar to a subset of those that occur in a constant environment. Thus, although pleiotropic networks are often considered to negatively affect adaptability, our results show that linkage of traits due to pleiotropy can result in the emergence of environmentally robust phenotypes during evolution in a non-complex environment.

Tuning of flower colours to the visual systems of pollinators

C.J. van der Kooij, J.T.M. Elzenga and D.G. Stavenga

Groningen Institute for Evolutionary Life Sciences

Plants attract pollinators by displaying colourful flowers. The coloration of flowers is due to reflection of light by structures inside the flower and filtering of reflected light by floral pigments. Floral visual signals comprise several components, such as hue, brightness and saturation, that are generated by specific anatomical structures and pigmentation. A challenge for modern studies on plant-pollinator co-evolution is understanding which perceptual factors are biologically relevant for plant reproduction, and how these colour signals evolved with respect to the visual system of pollinators. For example, bees, birds and butterflies have very different visual systems and will thus impose different selective pressures on floral visual signals. In this talk, I present results from an Angiosperm-wide comparative study on the optical and anatomical characteristics of flowers. Using a combination of optics, anatomy and evolutionary modelling, I show how floral visual signals are tuned so to yield strong stimuli to pollinators.

Towards a better understanding why plants became woody during evolutionary history

Frederic Lens

Naturalis Biodiversity Center

Woody plants (trees and shrubs) cover 30 percent of the planet's land area and offer crucial ecosystem services. It is unclear, however, why some plants are woody and others herbaceous. To this day, scientists have failed to provide satisfying answers, probably because wood formation is a complex process that can be triggered in different and largely understudied ways. As a first essential step to solve this evolutionary puzzle, I have compiled a database including nearly 7000 derived woody species – representing over 600 independent evolutionary transitions from herbaceousness towards (derived) woodiness. My database shows that these species mainly occur in continental regions with a marked drought period, and a number of hydraulic experiments in stems have supported the novel idea that derived woody species are more drought resistant than their herbaceous relatives. The hundreds of independent transitions suggest that the genetic mechanism leading to woodiness should be relatively simple, which is supported by a shrub-like woody mutant of *Arabidopsis* in which two flowering time control genes are knocked out. Outcomes of this project will revolutionize our understanding of wood formation, one of the most influential evolutionary innovations on earth.

Early Cretaceous origin of pteropods suggests their resilience to ocean acidification

K.T.C.A. Peijnenburg, A.W. Janssen, E. Goetze, D. Wall-Palmer, A.K. Burrige, J. Todd and F. Marlétaz
Naturalis Biodiversity Center

Pteropods (planktonic gastropods) are used as biological indicators to monitor the impact of ocean acidification because they live in the open ocean and have thin shells of aragonite, a metastable form of calcium carbonate. To understand the response of pteropods to previous changes in ocean chemistry, and thus predict their response to current climate change, we need a robust understanding of their evolutionary history. Here, we used a phylogenomic dataset (2654 genes, 28 taxa) and fossil evidence to resolve the phylogeny and timing of pteropod evolution. In agreement with the traditional taxonomy, we found a division between Thecosomata (mucus web feeders) and Gymnosomata (active predators). We estimated that these groups diverged in the lower Cretaceous and, therefore, both survived major global change events, such as the Paleocene Eocene Thermal Maximum, the closest analogue to modern-day ocean warming and acidification. These results suggest potential resilience of aragonitic calcifiers to ocean acidification.

The feedback between selection and demography shapes coevolutionary genomic change

Cas Retel

Eawag

Species interactions and coevolution are integral to ecological communities, but we lack empirical information on how they generate and purge genetic diversity. Understanding how biotic selection together with demographic processes shapes genomic variation is particularly interesting when adaptation occurs on ecologically relevant timescales. We use an experimental setup where host (*Chlorella*) and virus (*Chlorovirus*) populations reciprocally evolve resistance and virulence over 90 days. The temporal dynamics of population size and phenotypic change closely match across multiple replicates of co-evolution. They initially reflect an "arms race" with strong bottlenecks and growth phases, then change into a state where both species occur at stable densities, but phenotype abundances within populations fluctuate.

We now followed the accompanying genetic change, with temporally resolved whole-genome data for both species, which allowed us to identify selective sweeps in both species. These sweeps (reductions of genetic diversity due to strong positive selection) were accompanied by population growth, which interestingly facilitated the rapid build-up of genetic diversity in the host, consistent with neutral expectations. We further reveal differences between the species in the dynamics of molecular change. Hence, both eco-evolutionary feedback dynamics and interspecific differences in how genetic diversity is generated shape molecular evolution under species interactions.

Evolution of chromosomal coexistence

Shraddha Shitut, Daniel Rozen, Dennis Claessen and Alexander Kros
Origins Center

Evolutionary change within and between species arises gradually via slow accumulation of mutations. However, large changes via so-called major transitions can give rise to fundamentally new forms of organismal complexity, including complex cells, multicellular organisms, or societies. However, the mechanisms underlying these transitions remain poorly understood. Our aim is to understand the transition from simple to complex cells containing multiple, functionally interdependent, chromosomes. These chromosomes must coexist within cells and also persist during the origin of new cells, by evolving mechanisms to reduce segregational loss. Using cell-wall-deficient forms (L-forms) of the Streptomyces, *Kitasatospora viridifaciens*, we can potentially follow the evolution of chromosomal coexistence and segregation. In contrast to the mycelial morphology of wild-type streptomycetes, L-forms consist of unitary cells that lack a defined shape and divide independent of the conserved bacterial cell-division machinery. Preliminary experiments show that L-form cells can be easily fused to form polyploid cells containing multiple chromosomes. By using synthetically engineered hybrid cells containing multiple chromosomes that coexist via metabolic complementarity and a division of labor, we aim to examine the short and long-term evolutionary interactions between coexisting chromosomes. Experimental evolution of such fused hybrids will allow us to elucidate the dynamics, stability and gene

Evolutionary history of Polyneoptera and its implications the evolution of winged insects

Sabrina Simon

Wageningen University & Research

Polyneoptera comprises one of the four major subgroups of the winged insects, including 10 traditional orders with well-known and ecologically important groups such as termites, grasshoppers or stoneflies. However, the evolution of this group concerning relationships and various important phenotypic traits is unresolved. These ambiguities, which are considered as one of the major problems of insect evolution, do not only affect Polyneoptera itself but also have consequences for our general understanding of the early evolution of winged insects.

We provide the first comprehensive evolutionary picture of Polyneoptera by using a newly generated solid phylogenomic tree to trace the evolution of shape, habitat, and social behavior. Based on these results, we reconstructed and illustrated the last common ancestor of Polyneoptera. Our results imply that winged insects were terrestrial in all developmental stages, which implies that wings did not evolve in water. In addition, our study reveals that the highly specialized polyneopteran groups we observe today are derived from a largely unspecialized insect with many ancestral traits. Different forms of social behavior, changes in lifestyle, and associated morphological specializations evolved multiple times within Polyneoptera. The publication was an international collaborative study within the 1KITE project (1,000 Insect Transcriptome Evolution, www.1kite.org) and published on PNAS 2019, DOI: 10.1073/pnas.1817794116

Epigenetic inheritance of environmental effects in asexually reproducing plants

Koen Verhoeven

Netherlands Institute of Ecology

Epigenetic mechanisms such as DNA methylation can mediate stable, long-term responses to environmental stimuli. Transgenerational stability of such environment-induced epigenetic modifications is counteracted by epigenetic resetting that takes place in germlines. However, many plant species and crops reproduce asexually, either facultative or obligate, and bypass male germlines (apomixis / parthenogenesis) or both male and female germlines (vegetative propagation) between asexual generations. This sets the stage for transgenerational stability of epigenetic modifications. However, the epigenetic contribution to heritable trait variation and divergence within asexual plant lineages remains largely unknown. We use apomictic dandelion and clonal duckweed as models to evaluate heritable epigenetic variation within asexual plants. Using a reduced-representation bisulfite sequencing approach, we tracked DNA methylation variation across multi-generational experiments. Results show that transgenerational stability of DNA methylation variants can be substantial under asexual plant reproduction, but stability differs between asexual reproduction types, plant genotypes, and genomic contexts of DNA methylation. These results provide basic insight in the potential of DNA methylation to mediate heritable trait variation and adaptation in asexual plants.

Population size and the repeatability of antibiotic resistance pathways

*Martijn Schenk, Mark Zwart, Sungmin Hwang, Joachim Krug and Arjan de Visser
Wageningen University*

Mutations with either high rates or large fitness benefits may cause parallel evolution. I will present recent work, where we examine how population size mediates the relative contribution of mutation and selection in bacterial populations adapting to a novel antibiotic. Large populations show overall more parallel mutations than small populations. However, we find distinct roles for different mutational classes, with more parallel SNPs in large populations and more parallel chromosomal rearrangements in small populations. A trade-off between rate and benefit of mutations from these two classes underlies this pattern, causing rare large-benefit SNPs to win the competition from common small-benefit rearrangements in large but not in small populations. Our findings highlight the fundamental role of mutational bias also when selection is strong, and show the fundamental role of population size in mediating the contribution of different mutation classes to parallel evolution.

Eco-evo-devo of insect embryology

Chris Jacobs, Shixiong Cheng and Maurijn van der Zee
Leiden University

Insects offer great study systems for functional comparative developmental biology. Amongst other models, we use the flour beetle *Tribolium castaneum* and compare its early developmental patterning network to the fruit fly *Drosophila melanogaster*. In this way, the origin and evolution of hubs in the *Drosophila* network (such as Bicoid and Dorsal) have been elucidated. The *Drosophila* patterning system instructs the absence of an extraembryonic epithelium, the serosa, that secretes a protective cuticle in other insects. This evolutionary loss might have increased developmental speed. To accurately investigate trade-offs involved in increased developmental speed, we have experimentally selected replicate outbred populations of *Tribolium castaneum* for fast and slow embryonic development. We find that desiccation resistance and immune defense trade off with developmental speed. Pooled resequencing data of these *Tribolium* populations suggest that variation in laccase, a gene involved in serosal cuticle melanization and sclerotization, was a major target of selection. Preliminary data on these selection lines will be presented.

Taken together, we have begun to correlate microevolutionary selective forces to macroevolutionary changes in early developmental patterning networks. *Drosophila* underwent dramatic change; and developmental speed, desiccation resistance and immune defense seem to have been pivotal players.

Parental care patterns are associated with mating opportunities in the Chinese penduline tit *Remiz consobrinus*

Jia Zheng, Jan Komdeur, Tamás Székely, Donglai Li and Zhengwang Zhang
University of Groningen

Parental care is one of the most diverse social behaviours, since the type and duration of care and the sex of the care provider may vary both within and between species. Theoretically, by caring for or deserting the offspring the parents are expected to trade-off the benefits of offspring provisioning and thereby increase their survival against costs such as lost mating opportunities. Although theoretical studies have emphasized the significance of mating opportunities influencing parental decisions, the empirical evidence from wild populations is scanty. Here, we investigated the Chinese penduline tit *Remiz consobrinus*, a passerine bird that exhibits four types of parenting within a single population: biparental care, female-only care, male-only care and biparental desertion. We showed sexually divergent parental care patterns in this species, since nests built early in the season are more likely be deserted by males and cared for by females, whereas late nests are cared for by males and deserted by females. Furthermore, we provide evidence that the shift from deserting to caring by males is associated with seasonal declines in mating opportunities, since late-breeding males take a significantly longer time and experience a lower probability of finding a mate than early-breeding males. We also suggest male and female Chinese penduline tits are making parental care decisions independently, regardless of their partner.

Contributed Poster List

(Abstracts below)

Session I, 13.15-14.30: **odd numbers**

Session II, 17.20-18.30: **even numbers**

Poster pitches 10:30-10:45 (odd numbers): Melanie Lindner, Naomi Zweerus, Paula Ramos-Silva, Jeroen Koomen, Ken Kraaijeveld, Meike Wortel

Poster pitches 16:45 – 17:00 (even numbers): Kasper Hendriks, Marieke Jeuken, Kimberley Lemmen, Timo van Eldijk, Vicencio Oostra

1. Wei Zhang, NIOO-KNAW

Phylogenetic, morphological and ecological divergence in *Brachionus calyciflorus*, a newly described hybridizing species complex.

2. Kasper Hendriks, RUG

Phylogeography of Bornean land snails suggests long-distance dispersal as a cause of endemism.

3. Hylke Kortenbosch, RUG

Communities within communities: exploring the interaction between tropical land-snails and their microbiome communities.

4. Bertus Beaumont, TU Delft

Modular experimental evolution in the bacterial flagellar motor.

5. Raoul Van Oosten, VU

How organisms change their own selection pressure.

6. Emily Burdfield-Steel, UvA

Coping with change – how can chemical communication systems overcome disruptions?

7. Pieterella Luttkhuizen, NIOZ

Teaching evolution using a card game: negative frequency-dependent selection.

8. Aparajitha Ramesh , RUG

Should I stay or should I go? – Consistent individual differences in migration tendency.

9. Melanie Lindner, NIOO-KNAW

Changes in DNA methylation in relation to timing of reproduction in the great tit (*Parus major*).

10. Elise Fruitet, UvA

Evolution of dual-function sexual attractants.

11. Bernice Sepers, NIOO-KNAW

Epigenetics of animal personality: DNA methylation and its influence on exploratory behaviour in great tits (*Parus major*).

12. Langqing Liu, WUR

Unrevealing Conservation Statue of The Endangered Pygmy Hog (*Procula salvania*) Using Genomic Information.

13. Naomi Zweerus, UvA

Cracking the code of mutual signalling.

14. Marieke Jeuken, WUR

Multiple Dobzhansky-Muller incompatibilities contribute to reproductive barriers between lettuce species.

15. Jon Laman, RUG

Dietary modification reduces multiple sclerosis-like disease in adult marmoset monkeys.

16. Steven Declerck, NIOO-KNAW

Rapidly evolving zooplankton in a salinizing world: to what extent does adaptation to one salt increase tolerance to another one?

17. Thomas Blankers, UvA

Co-evolution of male and female sex pheromone communication under artificial selection in the Noctuid moth *Heliothis subflexa*.

18. Kimberley Lemmen, NIOO-KNAW

Experimental evolution supports predictions of the Growth Rate Hypothesis at the intraspecific level, but only in phosphorus rich environments.

19. Leila Iñigo de la Cruz, TU Delft

How does the recovery-trajectory from a nearly lethal mutation via experimental evolution affect the accompanying genetic interaction maps?

20. Thijmen Breeschoten, WUR

Influential meal: transcriptional diversity in the generalist *Spodoptera exigua*.

21. Lisette Mekkes, Naturalis/UvA

Pteropods make thinner shells along a natural ocean acidification gradient.

22. Anne-Marie Veenstra-Skirl, RUG

Characterization of controllable mutator strains.

23. Reza Ranjbar Choubeh, TU Delft

Quantitatively predicting the Genotype-Phenotype map for an evolutionary trajectory in budding yeast bottom-up.

24. Le Qin Choo, Naturalis

Insights into evolution in pteropods: obtaining genome-wide variation through target capture.

25. Paula Ramos-Silva, Naturalis

Wandering shells: the evolution of pteropod biomineralization.

26. Sarah Duxbury, WUR

Ecology and evolution of plasmid-mediated antimicrobial resistance (pAMR) transfer in the chicken microbiome.

27. Pedro Santos Neves, RUG

Does ontogeny matter? A simulation study on the effect of island ontogeny in the evolution of island clades.

28. Natalie van Dis, NIOO-KNAW

Winter moth (*Operophtera brumata*) adaptation to climate change: embryonic response to temperature.

29. Jeroen Koomen, WUR

Short term experimental evolution reveals the underlying mechanisms of multiple-stress resistance in a foodborne pathogen.

30. Elodie Wilwert, RUG

Visual plasticity - a driver of cichlid divergence?

- 31. Ken Kraaijeveld, VU**
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Experimental evolution using microbial communities shows divergence in community species composition and conversion in overall community metabolic p

*Anna Alekseeva, Joost van den Heuvel, Anneloes Groenenboom, Eddy Smid and Sijmen Schoustra
Wageningen University & Research*

Natural microbial ecosystems harbour diverse species and are stabilised through various interactions. However, theoretical predictions on the role of functional interactions for the ecosystem have not been rigorously tested. In most ecosystems these interactions are too complex to study in a controlled setting. Here, we use simpler microbial communities of Mabisi, a Zambian fermented milk product, to map the ecological niches. Using this system, we studied the mechanisms underlying eco-evolutionary stability in microbial ecosystems during long-term co-existence. We combined metatranscriptomic data from Mabisi samples before and after experimental evolution with GC-MS analysis of volatiles that Mabisi community produces. Unlike many milk products, both unpropagated and propagated Mabisi contain more than 50 genera of microorganisms, namely, bacteria and yeasts, as well as viruses. Gene expression in all sample profiles are similar to the ones found in natural microbial communities residing in a stationary growth phase, i.e. carbohydrates utilization, protein/fat metabolism, volatile production and cell defence. Replicate communities have diverged from each other during the propagation, indicating the establishment of distinct communities by the end of experiment. However, these diverse end-communities display a similar functional portrait on the level of transcriptional activity and aroma production. This indicates the divergence in the composition and the convergence in the

The effects of parasites upon non-host predator avoidance behavior in native and invasive gammarids.

Sajad Ashghali Farahani, Ido Pen, Per J Palsbøll and Jan Komdeur
Gelifes/RUG, Groningen

The life cycle of many parasites depends upon the successful transmission between successive hosts. Consequently, parasites are subject to selection upon traits that facilitate transmission between hosts. One such example is *Polymorphus minutus*, an acanthocephalan parasite, which alter the behavior of their intermediate hosts, gammarids, to facilitate the transmission to its final hosts, water fowls. A fundamental question is whether this ability of *P. minutus* to alter the intermediate hosts' behavior has evolved to specifically target sympatric gammarids, or gammarids in general? The effect of *P. minutus* in terms of avoidance towards a non-host predator, three-spined sticklebacks, *Gasterosteus aculeatus*, was assessed in three gammarid species; two native species *Gammarus pulex* and *Gammarus fossarum*, and an invasive species *Echinogammarus berilloni*. The degree of avoidance was assessed in choice experiments and revealed no differences in avoidance between infected and uninfected gammarids. In contrast, uninfected native gammarids showed a high degree of non-host predator avoidance behavior. This finding suggested that infection by *P. minutus* altered the predator avoidance in native *G. fossarum*, in a manner that increased the probability of transmission to water fowl, their final host. No such effect was observed in either *E. berilloni* nor *G. pulex*, suggesting that the ability by *P. minutus* to alter the host behavior has evolved in sympatry with host gammarid species. The results may also have implications in terms of the effects of parasites on the success or failure of invasive species.

Kept in the Dark for Far Too Long: Light Perception in Termite-associated Fungi

Ben Auxier, Lennart van de Peppel and Duur Aanen

Wageningen University

Kept in the Dark for Far Too Long: Light Perception in Termite-associated Fungi

Light is a fundamental stimulus, affecting growth, development, and reproduction in a myriad of ways. While it may seem that fungi grow blindly through their substrate, they actually use a sophisticated set of light-sensing proteins to determine the boundary of their substrate, and to regulate growth with the environment. However, members of the fungal genus *Termitomyces* has lived underground in obligate symbiosis with fungus-farming termites for ~30 million years. While it is apparent that underground termites have no eyes (except reproductive alates), it is unknown if their fungal symbiont is also blind. Based on initial genomic analyses, these fungi appear to lack functional photoreceptors, making them blind, while their non-symbiotic sister group have functional photoreceptors. We plan to test this using population genetic principles. This extended darkness may have allowed pseudogenization of the photoreceptors, and sequencing alleles from the population could show evidence of random drift. If these photoreceptors are pseudogenes, we expect mutations of conserved amino acid residues and excessive stop codons. We will also sample sister groups not living with termites, to test for functional receptors. While the genetic causes of blindness in cave fish, bats, and rodents are well studied, blind filamentous fungi are undocumented.

Examining these dynamics in a fungal system will help us understand why fungal blindness is much rarer than in animals.

Modular experimental evolution in the bacterial flagellar motor

Régis Flohr, Magda Pieczynska, Thierry Janssens, Min-Young Heo, Francesco Pedaci and Bertus

Beaumont
TU Delft

Life as we know it relies on complexes of interacting proteins that perform vital subcellular functions. Although comparative analyses have shown that the origin and innovation of protein complexes involved the integration of foreign subunits, direct insight into the capacity of evolution to achieve this is lacking. We used the bacterial flagellar motor as an experimental model to probe the integration of foreign orthologous subunits in an existing complex. On this poster, we present results that capture the evolutionary integration of incompatible orthologs along parallel step-wise mutational trajectories. The observed capacity evolution to resolve conflicts at and beyond the compatibility horizon likely facilitated the modular modification of protein complexes in nature.

babette: Bayesian phylogenetic inference from R

Richel J.C. Bilderbeek

University of Groningen

BEAST2 is a popular and powerful Bayesian phylogenetics tool, associated with a beginner-friendly GUI to help set up an inference model. For those that need to investigate many models, this approach is cumbersome. babette is an R package to call, run and read the results from BEAST, allowing for scripted use.

Co-evolution of male and female sex pheromone communication under artificial selection in the Noctuid moth *Heliothis subflexa*

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The phenotypic and genetic response to sexual selection, which can lead to conflicts between male and female traits and between reproduction and survival, is central to our understanding of the diversity in animal communication behavior. On the one hand, sexually selected traits are often dimorphic between the sexes, with males and females expressing strikingly different phenotypes despite sharing a genome. On the other hand, monomorphic traits, such as those used in bidirectional mate communication, experience sex-specific selection and can respond differentially to selection. Sexual signals may also be costly and subject to energy limitations. We have set up selection lines targeting acetate levels in the female *Heliothis subflexa* sex pheromone. Acetate levels vary within and between species and play a role in mate choice and behavioral isolation. Acetates are also potentially costly to produce. We investigate variation in the female pheromone, the biochemically linked male pheromone, and in the male response to female pheromones as a result of selection for increased or decreased levels of acetates. We estimate genetic variance (heritability) and covariance across these traits in a control line and track phenotypic changes in the high and low selection lines. Ultimately, we will test how (artificial) selection of the female pheromone has affected the mean and variance, and the genetic (co)variances in and among co-evolving sex pheromone communication traits.

Physical linkage and directional mate preferences generate linkage disequilibrium for behavioral isolation

*Thomas Blankers, Emma L. Berdan, Frieder Mayer and R. Matthias Hennig
University of Amsterdam*

Behavioral isolation is a potent barrier to gene flow and a major source of animal diversity. However, to establish and maintain behavioral isolation, linkage disequilibrium (LD) between sex-specific traits is required. Theoretical research suggests stringent conditions under which LD can be maintained, but there is limited empirical insight into the mechanisms coupling sex-specific traits. I will discuss recent insights into male song and female preference co-evolution in the field crickets *Gryllus rubens* and *G. texensis*. These closely related species diverged with gene flow and there is strong positive genetic covariance between the highly divergent male song and female preference traits. New results show that this covariance is in part explained by partial physical linkage. Both quantitative trait loci for male and female phenotypes (pQTL) as well as QTL for gene expression associated with those phenotypes (eQTL) overlapped, thus coupling male and female traits. However, major effect loci for sex-specific traits were never on the same chromosome. The finely-tuned, highly divergent preference functions are likely an additional source of LD between male and female traits. Together, these findings reveal in detail the joint contribution of mate preferences and physical linkage to maintaining LD and to facilitating co-evolution in the face of gene flow.

The egg effect: Egg size determines chick quality in Eurasian Jackdaws *Corvus monedula*

Mirjam J. Borger, Christina Bauch, Jelle J. Boonekamp and Simon Verhulst
University of Groningen

Variation in rearing conditions is known to affect fitness in early and later life, but the underlying mechanisms of this relationship remain elusive. In a previous study on jackdaws (*Corvus monedula*) we found a correlation between egg size and nestling quality until fledging, which showed that egg size is an important rearing condition in this species. However, how exactly egg size causes effects on offspring performance remains unclear. Our research aims to unravel the correlation between egg size and nestling mass (a quality marker), by testing if i) egg size itself or ii) the shared parental or environmental quality, explained the observed correlation. We cross-fostered complete clutches of Jackdaws to disentangle the effects of parental care and of the egg itself on nestling mass on day 5, which correlated with the survival probability until fledging. We found that the correlation between egg size and day 5 mass was explained only by egg effects. Intriguingly, the slope of the correlation between egg size and day 5 mass is steeper (1.7 g/cm^3) than the slope between egg size and day 1 mass (0.7 g/cm^3). No effect was found of egg size on incubation time, so slope differences could not be caused by a longer development time and therefore being further on the exponential growth curve. Thus our results could indicate that nestlings from larger eggs grow faster in the egg, or that egg size has carry-over effects on the growth rate of nestlings.

Influential meal: transcriptional diversity in the generalist *Spodoptera exigua*

Thijmen Breeschoten, Vera I. D. Ros, M. Eric Schranz and Sabrina Simon
Biosystematics group, WUR

Insects and plants have co-evolved for over 420 million years. Insect herbivores have evolved mechanisms to cope with plant defences and are often adapted to feeding on a restricted range of host-plants. Yet, many species are considered generalists which are facing a large variety of host plants and defences. De novo transcriptome sequencing in insect herbivores provide insights into molecular and evolutionary mechanisms of plant use and host plant specialisation. Recently we have generated gene expression data of the generalist moth *Spodoptera exigua* (Noctuidae: Lepidoptera) feeding on different hosts. By comparing the gene activity of *S. exigua* while feeding on different hosts we are able to study shared and different gene expression patterns providing insights into molecular and evolutionary mechanisms of herbivory.

Coping with change – how can chemical communication systems overcome disruptions?

Emily Burdfield-Steel and Astrid Groot

University of Amsterdam

In many insect species females attract mates via long-range pheromones. This form of chemical communication often depends on fixed proportions of different compounds in order to attract males of the correct species. As the larval of such insects are often pest of crops, this mode of signaling has been exploited for pest control through the use of artificial pheromones. This is thought to avoid many of the pitfalls of insecticide use, as it is safer and more species –specific. However, the widespread use of such techniques is likely to create strong selection on target species. The long term success of these methods relies on us having a good understanding of the potential for these communication systems to respond to change. One way to achieve this is to study how species have adapted to a more historical challenge- other species. Reproductive interference occurs when interactions between species during the process of mate acquisition or mating itself result in a cost for one or both species. Of all forms of reproductive interference, signal jamming- where the sexual signals of one species interfere with those of another, is likely to be the most common. By examining the ways in which a pheromone-producing moth species responds to the threat of reproductive interference we can gain insights into how such species can alter their communication systems to overcome disruption.

Insights into evolution in pteropods: obtaining genome-wide variation through target capture

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Pteropods are bio-indicators of ocean acidification, hence it is important to understand their adaptive potential by studying their spatial scale of genetic variation among populations. Pteropods are non-model organisms with only a few molecular markers and 11 draft transcriptomes as genetic resources. We assembled a draft genome of the species *Limacina bulimoides* as it is a common species found in the tropics and subtropics worldwide. This draft genome consisted of 3.8 million contigs with a total size of 2.9 Gbp, representing approximately 60.2% of its actual size. We used this new genome, together with a transcriptome and a few known genes to design a set of capture probes, to investigate genome-wide variation across multiple individuals of *L. bulimoides*. In total, we chose 2,899 targets and designed corresponding capture probes. Most of these were recovered by the first capture reaction performed on the targeted species. The application of these capture probes to four other species of limacinids related to *L. bulimoides* showed an exponential decrease in the number of SNPs obtained for each species with increased genetic distance from the initially targeted species. These newly developed genomic resources represent invaluable tools for studying patterns of variation, historical demography and outlier SNPs in *L. bulimoides*. We propose that designing multi-species capture probe sets would likely improve the capture efficiency on a set of genetically close species.

Quantitatively predicting the Genotype-Phenotype map for an evolutionary trajectory in budding yeast bottom-up

*Reza Ranjbar Choubeh, Werner Daalman and Liedewij Laan
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For budding yeast, rapidly choosing the new bud direction (polarization) is essential for fitness. About a dozen core proteins are involved, yet predicting mutant phenotypes is complex due to their rich interaction network. However, by coarse-graining these molecular details to the concentration and dynamics of the core polarity protein Cdc42 in the cell, we manage to quantitatively map polarity mutants to measured growth rates, using a parsimonious model for cell growth and protein turnover. We will further test this model through live cell microscopy on cells with a fluorescently tagged Cdc42, to directly relate the Cdc42 concentration and dynamics to cell fitness in wild-type and mutants during budding. This comparison will provide direct evidence to rule out or confirm our model about (the evolvability of) cell polarization in budding yeast.

When nature strikes – Species extinction or adaptation?

*Aleksandra Cvetkoska, Friederike Wagner-Cremer, Sinead Collins and Dedmer B. Van de Waal
Netherlands Institute of Ecology (NIOO-KNAW)*

Global environmental change imposes a major threat to Earth's biodiversity. In a rapidly changing world, survival of organisms will largely depend on their ability to adapt. Despite growing knowledge of species responses to short-term environmental change, there is a lack of long-term experimental data to assess adaptive responses, and test for the potential for rapid evolution. Due to their long existence and exceptional biodiversity, ancient lakes are models for studying the evolutionary consequences of long-term global change. The oldest and most biodiverse European lake, Lake Ohrid, offers an exclusive setting to link environmental changes and eco-evolutionary patterns in different groups of organisms. The 600m core recovered from the lake in 2013 is unique by its continuous preservation of diatoms, throughout its ~1.4 million years lake history. This diatom record provides clear evidence for evolution within the dominant planktonic genera, *Cyclotella* and *Pantocsekiella*. Pilot data highlight the extraordinary morphological variability within some of the species, which is likely driven by environmental changes. However, the relative contribution of phenotypic plasticity versus adaptive evolution in promoting diatom phenotypic diversity cannot be determined from the fossil record alone. We therefore will integrate experimental evolution and Quaternary paleoecology, to show the joint contributions of plasticity and evolution on phenotypic diversity in diatom populations.

Rapidly evolving zooplankton in a salinizing world: to what extent does adaptation to one salt increase tolerance to another one?

Steven Declerck, Shuai-Ying Zhao and Libin Zhou

NIOO-KNAW

Rapid evolutionary adaptation is a mechanism through which populations of aquatic organisms may be able to cope with the increasing levels of salinity associated to climate change. Salinity stress does not only involve increased osmotic pressure but, depending on salt chemical identity, also toxicity by salt ions. We tested (1) the capacity of populations of the freshwater rotifer *Brachionus calyciflorus* to rapidly adapt to increased salinization, (2) if salts differ in toxicity and (3) if evolutionary adaptation to one type of salt alters the potential to cope with other types of salts? We performed a laboratory evolution experiment subjecting replicate populations to low and high NaCl treatments. Subsequently, in a common garden experiment, populations from the evolution experiment were exposed not only to NaCl but also to CaCl₂ and NaSO₄. In each of these treatments, salt levels were gradually increased until populations realized zero net population growth and the corresponding osmolarities were calculated. Comparisons of these osmolarities between salt treatments show that adaptation to NaCl increases the ability of populations to cope with toxicity due to calcium but not to sulphate ions. To conclude, our results suggest that in a metapopulation context evolutionary adaptation to one salt type may facilitate colonization of patches that are salinized by other types of salts, but this outcome will largely depend on salt chemical identity.

Evolutionary dynamics of Virtual Microbes: Anticipating a serial transfer protocol together or alone

Bram van Dijk, Jeroen Meijer and Paulien Hogeweg

Utrecht University

Experimental evolution of microbes often involves a serial transfer protocol, where microbes are repeatedly diluted by transfer to a fresh medium, starting a new growth cycle. Here we study how in silico evolved Virtual Microbe "wild types" (WTs) adapt to a serial transfer protocol to search for generic evolutionary features, and to investigate how these features depend on prior evolution. We show that all WTs adopt a fine-tuned balance of growth and survival, anticipating the regularity of the serial transfer protocol. We find that this anticipation can happen either by means of a single lineage, or by multiple ecological lineages that specialise either on the growth phase or the stationary phase. Interestingly, replicate populations of the same WT initially show similar trajectories, but may subsequently diverge along a growth rate versus yield trade-off. Taken together, we find that all our in silico WTs show the same anticipation effects – fitting the periodicity of a serial transfer protocol – but prior adaptations can strongly determine the details of subsequent evolution. Our results reveal important insights into the dynamics and relevant selection pressures in experimental evolution, advancing our understanding of the eco-evolutionary dynamics of microbes.

Winter moth (*Operophtera brumata*) adaptation to climate change: embryonic response to temperature

N.E. van Dis, M. van der Zee, R.A. Hut, M.E. Visser and B. Wertheim
NIOO-KNAW; RUG

Climate change influences the seasonal timing of many organisms, but at different rates such that mismatches can occur between trophic levels. Increased winter temperatures caused winter moth egg hatching to advance, leading to a mismatch in timing between winter moth caterpillars and their food source young oak leaves. In response, winter moth egg development has genetically adapted to climate change. Eggs now need higher temperatures to complete development at a given time compared to 10 years ago, leading to a better match with oak bud burst. To investigate how and when temperature influences embryonic development, an experiment was carried out. The aim of the experiment is two-fold, to determine (1) which stages of winter moth embryonic development can be distinguished, and (2) the effect of temperature in- or decreases on developmental rate in these different stages of development. In a split-brood design, sub-clutches of eggs were transferred from a baseline temperature of 10 degrees to a colder or warmer temperature for two weeks at different times during development. Before transfer and two weeks after transfer, eggs were fixated and imaged using confocal fluorescence imaging. Here we present the first glimpses of winter moth embryonic development, a moth with an unusually long development of multiple months, and one of the few species that has been able to rapidly adapt to climate change.

Host control in the nitrogen fixing symbiosis

Simon Dupin

Kiers laboratory

Organisms rely on symbiotic associations for metabolism, protection, and energy. However, these intimate partnerships can be vulnerable to exploitation. What prevents microbial mutualists from parasitizing their hosts? In legumes, there is evidence that hosts have evolved sophisticated mechanisms to manage their symbiotic rhizobia, but the generality and evolutionary origins of these control mechanisms are intensely debated.

Here, we focus on the symbiosis between Parasponia hosts and N₂-fixing rhizobium bacteria.

Parasponia is the only non-legume lineage to have evolved a rhizobial symbiosis, and thus provides an important evolutionary replicate to test how rhizobial exploitation is controlled. A key question is whether Parasponia hosts can prevent colonization of rhizobia under high nitrogen conditions, when the contribution of the symbiont is nonessential.

We show that, like legumes, Parasponia was able to control nodulation, and this depended on exogenous nitrogen concentrations. We found evidence that Parasponia controls rhizobial fitness by mediating the amount of intracellular infection depending on ammonium nitrate availability.

Our results demonstrate that control mechanisms over rhizobia are not exclusive to the Fabales clade. These new insights in evolution of host control could help in understanding the evolution and stabilization of symbiotic interactions, more generally.

Ecology and evolution of plasmid-mediated antimicrobial resistance (pAMR) transfer in the chicken microbiome

Sarah Duxbury, Mark Zwart, Jesse Alderliesten, Egil Fischer, Arjan Stegeman, Arjan de Visser
Wageningen University

Plasmid-mediated antimicrobial resistance (pAMR) presents a major challenge to both human and animal health. Extended spectrum beta-lactamase (ESBL) resistance is particularly prevalent, with plasmids and resistance genes able to transfer from animal to human-associated bacterial isolates. The prevalence of pAMR is affected by selection pressures on host bacteria, compensatory adaptation of the host and plasmid and plasmid conjugation rates. Despite this, we know little about the ecological and evolutionary processes influencing the invasion and maintenance of pAMR within the microbiome of a (group of) individual animals. We study these processes using controlled experiments in the laboratory to measure conjugation rates, fitness effects of plasmid carriage and rates of host-plasmid coevolution, in the absence and presence of synthetic microbial communities. We use in vitro experiments for parameter estimation, focusing on between-*Escherichia coli* strain pAMR transmission of an ESBL gene that is highly prevalent in the chicken gut microbiome. To develop more realistic parameter estimates, we use chicken microbiome-relevant communities to study effects of resource depletion and metabolic bi-products on pAMR dynamics. In vitro estimates will be applied to mathematical models developed at Utrecht University, to better predict within-host transmission and evolution of pAMR.

Does evolutionary rescue theory predict the evolution of antibiotic resistance?

T.J.B. van Eldij and, F.J. Weissing

Groningen Institute for Evolutionary Life Sciences

Evolutionary rescue theory has the potential to help us better understand when to expect the evolution of antibiotic resistance in bacterial populations. However, current models of evolutionary rescue fail to account for the mechanisms deployed by bacteria to cope with stressful conditions (like the presence of antibiotics). By means of stochastic models, we address two of these mechanisms. First, we consider biofilm formation, which occurs in the majority of bacterial infections. By systematically changing the degree of population mixing (from a homogeneous culture to a biofilm), we investigate how the probability of evolutionary rescue is affected by spatial structure. It turns out that the probability of rescue is substantially lower under biofilm conditions. Second, we consider the formation of persister cells; dormant cells that tolerate antibiotics exposure. The simulations reveal that the formation of persister cells increases the probability of evolutionary rescue. However, the effect size is negligibly small unless the percentage of persister cells is unrealistically high. Findings like these help us to assess whether, and to what extent, evolutionary rescue theory is able to predict the evolution of antibiotic resistance in a clinical setting.

Evolutionary rescue theory, antibiotic resistance and the details of bacterial infection.

T.J.B. van Eldijk and F.J. Weissing

Groningen Institute for Evolutionary Life Sciences

Evolutionary rescue theory has the potential to help us better understand when to expect the evolution of antibiotic resistance in bacterial populations (i.e. probability of rescue). However, current models of evolutionary rescue fail to account for some of the more complex mechanisms underlying bacterial infections. To address this, I have constructed two stochastic models. Biofilm formation occurs in the majority of bacterial infections. Therefore, I studied how the presence of spatial structure (i.e. homogeneous culture vs biofilm) influences the probability of rescue. I show that the probability of rescue is significantly lower under biofilm conditions. Next, in a second model I studied how the formation of persister cells i.e. dormant cells that tolerate antibiotics exposure, influences the probability of evolutionary rescue. I show that the formation of persister cells does increase the probability of evolutionary rescue, yet this only occurs if the percentage of persister cells is unrealistically high. In realistic scenarios the formation of persister cells has no influence on the probability of rescue. These findings help us understand how some of the nitty-gritty details of bacterial infections, impact evolutionary rescue predictions; a key step in the application of evolutionary rescue theory to help address the evolution of antibiotic resistance in a clinical setting.

What if MacArthur & Wilson had used molecular phylogenies?

L. Valente, A.B. Phillimore, M. Melo, B. Warren, S. Clegg, R. Tiedemann, K. Ravenstein, JC Illera and R.S Etienne.

Groningen Institute for Evolutionary Life Sciences

In the theory of island biogeography, MacArthur & Wilson hypothesised that island biodiversity depends on island features such as area and isolation, due to their effect on the critical underlying processes of colonization, extinction and speciation. Testing this hypothesis requires information on the timing of species arrival and diversification for entire communities on islands. These can now be extracted from divergence-dated molecular phylogenies. We have put together the largest-ever global phylogenetic dataset of times of colonisation and speciation of birds on islands. We obtained DNA sequence data from the 581 taxa on 41 archipelagos, and their closest mainland relatives. We fitted a new version of our DAISIE model to this global phylogenetic dataset, where instead of estimating individual colonisation, speciation and extinction rates for each archipelago, we estimated the hyperparameters that control the relationship between these rates and island features (area, isolation) on a global scale. By bridging together the theoretical foundations of MacArthur & Wilson's model with the temporal information contained in molecular phylogenies, we are able to successfully reproduce global patterns of insular diversity including number of colonisations and radiations on different islands, as well as infer the shape of several key macroevolutionary relationships, such as the cladogenesis-area, extinction-area, colonisation-isolation and anagenesis-isolation relationships.

Evolution of dual-function sexual attractants

*Elise Fruitet, Thomas Blankers, David G. Heckel and Astrid T. Groot
IBED, UvA*

For sexually reproducing species, it is important to find a mate and even better to find a good one. Sexual communication not only allows individuals to discriminate between potential partners, but also to discriminate between intra- and interspecific individuals and avoid hybridisation. Sexual attraction through sex pheromones is best studied in moths, where females emit species-specific pheromone blends. The species-specificity stems from the presence and ratios of the different components. Some components are not only attractive for potential partners, but also repellent for sympatrically occurring species, which is for example the case for acetate esters. How these dual-function pheromone components evolve is still a mystery.

This project aims to determine the genetic basis of and selection forces acting on the acetates in the noctuid moth *Heliothis subflexa*. Previous genetic analyses have identified two major QTL underlying acetate production and we are currently conducting functional analyses with candidate genes. Plasticity in acetate levels have also been found, depending on the chemical environment the females experienced. We are currently assessing the costs of producing these dual-function components by investigating a) the reproductive output of females with high and low acetate levels, b) whether females with high acetate levels are more or less susceptible to pathogens, and c) whether and when females with different acetate levels differ in their attractiveness.

Cheating in *Neurospora crassa* is caused by inactivating mutations of fusion genes

Alex Grum-Grzhimaylo, Eric Bastiaans, Joost van den Heuvel, Alfons Debets and Dur Aanen
NIOO-KNAW

Free access to costly cooperative actions should open the door to free-riders. This applies not only to human cooperation, but also to microbial cooperation. Experimental evolution of the fungus *Neurospora crassa* supports this general prediction: unrestricted fusion between individuals favours cheating mutants. Using whole-genome sequencing we show striking parallelism among those experimentally-evolved cheaters. Paradoxically, all eight have unique mutations in fusion genes, presumably leading to loss-of-function. We experimentally show that reduction of fusion is causal to cheating. Cheaters hardly fuse with cheaters but still at appreciable frequencies with wild type. The benefit of cheaters in the resulting chimera is due to disproportional representation in spores. Our results imply that fusion has costs, making it an act of altruism. Non-fusing cheater mutants can profit unilaterally, by limiting access to themselves, while gaining access to wild-type individuals, but only at low frequencies and at the cost of collective performance.

Phylogeography of Bornean land snails suggests long-distance dispersal as a cause of endemism

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University of Groningen

Islands are often hotspots of endemism due to their isolation, making colonization a rare event, and as such facilitating allopatric speciation. Dispersal usually originates from nearby locations according to a stepping-stone model. We aimed to elucidate colonization and speciation processes in an endemic-rich system of tropical land-based islands that does not seem to follow the obvious stepping-stone model of dispersal. We studied the phylogeographic relations within several island radiations of three unrelated species complexes of land snail, each common on limestone outcrops in the study region in Sabah, Malaysian Borneo. We found that most archipelagos have been colonized multiple times over the past three million years, from within the region. Gene flow in these Bornean land snails is rare and has been affected both by small-scale dispersal, where it leads to isolation-by-distance, and long-distance colonization. Our results demonstrate that long-distance dispersal, and resultant colonization, is actually relatively frequent and seems the origin of endemism in our study system. The formation of highly localized, isolated “endemic populations” appears to form the onset for a complex radiation of endemic taxa.

The role of epigenetic mechanisms in the evolution of cognitive traits

Krista van den Heuvel

NIOO-KNAW

Epigenetic processes may play an important role in the evolution of cognitive traits as they can provide an organism with the opportunity for an adaptive response to a change in the environment. To understand the role of epigenetics in shaping individual variation of and natural selection on cognitive performance, we must find out what kind of epigenetic variation is important for the evolution of cognition. However, studies on epigenetics and cognition have mostly focused on laboratory animals, thus we must extrapolate the current knowledge to an ecologically relevant system. Therefore, I will use the great tit (*Parus major*) as a model species to assess the role of epigenetic regulation in the evolution of cognitive traits.

Communities within communities: exploring the interaction between tropical land-snails and their microbiome communities

Hylke Kortenbosch

Groningen University

Communities are shaped by yet poorly understood processes like dispersal, drift and selection. Natural communities encompass more than just one taxonomic group or spatial scale, as do the forces that shape these communities. Microbiomes are an integral part of life, and also part of their host's community. They are often vital to their host's functioning and are continuously shaped by their host and its environment. It remains unknown, however, if the transfer of microbes between different host species in a community has significant effects on the microbiomes within said community. Such effects could have major implications for microbiome functioning, as well as microbiome phylogenies in relation to their host's. The aim of the study was to investigate microbiome structure and phylogeny across isolated host communities. Using a combination of Bornean microsnail community data and metabarcoding of bacterial communities, we showed that microbial communities diverge along with their host species. We showed that host species isolation is a stronger factor shaping microbiome composition than the community composition of related host species per location. Our results suggest that within-host species dispersal, selection and drift are stronger actors in shaping microbiome than dispersal or transmission of bacteria among host species. Our results underline microbiomes' potential as an additional source of information on their host's population dynamics and evolutionary history.

How does the recovery-trajectory from a nearly lethal mutation via experimental evolution affect the accompanying genetic interaction maps?

Leila Iñigo de la Cruz

Delft University of Technology

One of the biggest questions for cell biologists has been how to integrate different levels of organization in biological systems: from cellular functions to gene interaction networks, and viceversa. There is evidence, both from experimental evolution as from large scale comparative studies, that strains and species can perform core cellular functions with different sets of connected genes. For example, the evolutionary trajectory from a nearly lethal gene deletion (BEM1) in budding yeast reproducibly shows an almost complete recovery of the growth rate of the organism, through three subsequent loss of function mutations in the genes BEM3, NRP1 and BEM2. We ask: how is the gene interaction network perturbed in each of these losses ? With the advent of high throughput genetic techniques, it is possible to experimentally take a closer look at the dynamics of genetic interactions patterns for the subsequent steps of the evolution experiment. We will use a recently developed technique called Saturated Transposon Analysis in Yeast (SATAY). This technique allows a complete mapping of the yeast genome by random insertions of transposons at different genomic locations.

Multiple Dobzhansky-Muller incompatibilities contribute to reproductive barriers between lettuce species

Anne Giesbers and Marieke Jeuken

Wageningen University

Acquisition of reproductive isolation is a key step in speciation. Crossing species may result in a hybrid with reduced viability or fertility due to genetic incompatibilities between species, a phenomenon known as hybrid incompatibility (HI). An interspecific F1 hybrid of the self-fertilizing lettuce species, *Lactuca saligna* and *L. sativa*, showed normal viability but a severely reduced fertility of only 2% seed set. Despite this reduced fertility, we developed various types of mapping populations for a genetic dissection of HI. We determined the genome-wide distribution of HI in a set of introgression lines (ILs) with a single introgression segment from *L. saligna* introgressed into *L. sativa*. Almost all ILs contained homozygous introgression segments. For five ILs, however, we were only able to obtain a heterozygous introgression. Their inbred progenies displayed severe transmission ratio distortion (TRD) and complete non-transmission of the homozygous *L. saligna* introgression, i.e. absolute HI. These five HI might be caused by deleterious allele combinations at epistatic loci. We found epistatic loci by analysis of an F2 (from the selfed F1). Validation of interactive pairs was done by double-introgression progenies, which showed that three HI are explained by nuclear pairwise incompatibilities. One of the digenic HI showed 29% reduced seed set and its pattern of TRD pointed to a gametophytic barrier, surprisingly in both male and female gametophytes (sex-independent).

Mechanisms of hormone mediated parent-offspring conflict

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Embryonic exposure to maternal hormones can have profound consequences for its later phenotype. Bird species have been used extensively to study these effects, showing systematic variations in maternal hormone allocation to the egg. However, the embryo itself is often seen as a passive responder and its role in the evolution of parent-offspring conflict has largely been ignored. By focussing on early hormone dynamics in the egg and the embryo we found that (1) The active maternal hormones in the egg are substantially and rapidly metabolized by the embryo, this suggests that the embryo itself can time its exposure to the maternal signal; (2) The dynamics of androgen metabolism differs systematically between eggs of different laying order, this opens the possibility that the embryo can deal with the maternal signal depending on the context it experiences; (3) The embryo expresses steroid receptors in its extra-embryonic membranes even before its own hormone production starts, indicating that selection has favoured the early perception of the maternal signal; and (4) The embryo downregulates these receptors in response to increased egg steroid levels, indicating that the embryo can regulate the effectiveness of the maternal signal. Altogether, the results imply that the embryo can potentially play its own role in parent-offspring conflict, opening new avenues to integrate mechanistic, developmental and evolutionary approaches to the study of maternal effects.

Attachment forces of herbivorous snails on leaves of terrestrial and epiphytic orchids

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Naturalis Biodiversity Center

Plants protect themselves against herbivores with various structures embedded in their epidermis such as epicuticular hairs, waxes, glands and glandular trichomes. Despite the large variation in epi- and subcuticular protective structures, though, orchids suffer from herbivore damage, both in the wild as well as in cultivation. Invasive and exotic herbivorous snails are among the most common orchid pests and terrestrial orchids seem to suffer considerably more from snails than epiphytic species. To answer the intriguing question whether terrestrial and epiphytic orchids protect themselves differently against herbivorous snails, we investigate the adhesive forces of gastropod mantle feed of two very common orchid pests. By analysing the adhesion of small Subulinidae and larger Pleurodontidae snails with a 'bug centrifuge', we study how adhesive forces are related to the presence of epicuticular hairs, waxes, glands and glandular trichomes on the leaves of four different orchid species, two epiphytic ones and two terrestrial species. Anatomical details of the epi- and subcuticular protective structures present on and in the orchid leaves investigated are studied with Scanning Electron Microscopy (SEM) and Light -Microscopy (LM). Our study provides more insight in the various ways by which orchids protect themselves against herbivorous snails. This knowledge can help improve the conservation of endangered orchid species

Short term experimental evolution reveals the underlying mechanisms of multiple-stress resistance in a foodborne pathogen

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Listeria monocytogenes is a foodborne pathogen notorious for its robustness, and further insight into the mechanisms underlying its adaptive stress response and survival strategies is required.

Physiological characterization of two previously isolated multiple-stress resistant *Listeria monocytogenes* mutants with low growth speed revealed a tradeoff between multiple-stress resistance and growth speed. Short term evolution experiments with these mutants, one with an amino acid substitution in the ribosomal rpsU gene (Arg-Pro), and one with a complete deletion of rpsU, allowed selection of evolved strains with wild-type growth kinetics and loss of multiple-stress resistance. Whole genome sequencing of evolved strains suggests that compensation of the substitution was possible via an additional SNP in rpsU (either Pro-Thr, or Pro-His), while compensation of the deletion was achieved by SNP's in the ribosomal rpsB gene resulting in amino acid substitutions (arg-his, or arg-ser).

Additionally, transcriptome analysis, and sucrose density gradient centrifugation of the ribosomes were used to gain insight into the underlying mechanisms of ribosome-mediated multiple-stress resistance in this foodborne pathogen. These results can ultimately be used in evolutionary forecasting, while information on which mutations can affect pathogen fitness and stress resistance is essential for modelling the presence of *L. monocytogenes* in the food environment.

Episodic bursts of gene duplication, but no evidence for whole-genome duplications in Hexapods

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Gene duplication is a pervasive force in evolution. Gene duplicates can open new adaptive avenues by providing new genetic material that is largely free of functional constraints. Whole-genome duplications (WGD) provide large amounts of such raw material for evolution and have been important in the evolution of plants and, more rarely, in animals. Here, we examine patterns of gene duplication in the genome sequences of 19 Hexapod species, to evaluate the contribution of WGD in the evolution of this animal group. We find that some species retain relatively large numbers of gene duplicates, while others retain very few. In only two cases do we find substantial amounts of synteny among these retained duplicates, as would be expected under WGD. However, close examination of these genomes shows that for most syntenic blocks, the two duplicates are located on the same chromosome, often as palindromes. This is not congruent with WGD, where the duplicates would be on two separate chromosomes. Instead, we suggest that episodic activity of transposable elements may have caused large segmental duplications, which superficially resemble ancient WGD events. We conclude that gene duplication has been common in the evolutionary history of Hexapods, but that current genomic evidence does not support claims of past WGD events.

Dietary modification reduces multiple sclerosis-like disease in adult marmoset monkeys

J. Laman et al

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The interplay between diet, gut microbiota and immunity (gut-brain axis) is essential in the development of the demyelinating disease multiple sclerosis (MS). EAE in marmosets is a translationally relevant MS model. A new dietary supplement (yoghurt-based and vitamin-enriched) reduced EAE from 100 to 60%. Hence, we compared the new and classic dietary supplements in dizygotic twins. In the monkeys reverted to the classic diet a 100% EAE incidence was observed, versus 75% for the new diet. Demyelination in the spinal cord was significantly lower for the new diet and immunological inflammatory parameters were downregulated. Brain RNA-sequencing reflected reduced apoptosis and enhanced myelination in marmosets receiving the new dietary supplement. First-ever systematic typing of the marmoset gut microbiota using 16S rRNA sequencing demonstrated a unique, *Bifidobacteria*-dominated composition. In conclusion, dietary modification markedly reduces clinical disease and pathology of MS-like disease in adult, outbred, conventionally-housed marmosets, providing a new preclinical intervention model.

Experimental evolution supports predictions of the Growth Rate Hypothesis at the intraspecific level, but only in phosphorus rich environments

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In ecological stoichiometry, the Growth Rate Hypothesis (GRH) states the growth rate capacity of organisms is positively related to somatic P content. As consumers acquire P from food resources the degree to which enhancement of growth rate can occur should critically depend on resource P-content. Hitherto, evidence for the GRH has only been observational and based on interspecific comparisons. We investigated i) the applicability of the GRH predictions at an intraspecific level and ii) the influence of P- availability on the microevolutionary potential of animal populations to increase their growth rates. We subjected genetically diverse populations of the cyclical parthenogenetic microzooplankton *Brachionus calyciflorus* to r-selection environments with low or high P availability. Subsequently, we studied the evolutionary response of their population growth rate and somatic P-content in a fully crossed common-garden experiment. From this, we observed increased growth rates in populations adapted to HP and LP conditions, although the mechanisms through which they achieved this differed between selection regimes. In accordance with the GRH, HP-selected populations responded with increased body P-content. This was in sharp contrast with LP-selected populations who seemed to rely on alternative strategies. This study is the first to experimentally demonstrate that predictions of the GRH are applicable at an intraspecific level, however the GRH only holds when P is non-limiting.

Changes in DNA methylation in relation to timing of reproduction in the great tit (*Parus major*)

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Timing of reproduction has major fitness consequences and is phenotypically plastic, so that laying date adaptively shifts with annual environmental variation. A potential key mechanism underlying this plasticity is DNA methylation, which is known to alter gene transcription in response to environmental conditions. Here, we examine short-term changes in DNA methylation in female great tits (*Parus major*) using red blood cell samples collected repeatedly throughout the breeding season. We used a reduced representation bisulfite sequencing approach, and the resulting methylation call information, to identify differentially methylated sites (DMS) between four reproductive stages within the breeding season. Our most promising findings include a gene encoding for Steroidogenic factor 1 (NR5A1) with three DMS within its promoter region. All three DMS showed low methylation level at the time females were laying and a clear increase in methylation level afterwards, consistent with a potential downregulation of NR5A1 after egg-laying. Our findings indicate that short-term changes in DNA methylation are involved in timing of reproduction in great tits. We currently investigate how such changes in blood DNA methylation patterns relate to changes in gene expression of tissues known to regulate timing of reproduction (i.e. hypothalamus, ovary, liver) to better understand the functional role of changes in blood DNA methylation on seasonally expressed phenotypes.

Unrevealing Conservation Statue of The Endangered Pygmy Hog (*Procula salvania*) Using Genomic Information

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Conservation breeding is becoming an increasingly important tool to prevent extinction. This by providing organisms for reintroduction to either re-establish or wild populations in their natural environment, or for assisted colonization outside of historical natural ranges by species translocation. The pygmy hog (*Procula salvania*) is the smallest and the rarest wild suid in the world. Once thought extinct, it has been classified as a critically endangered species according to the International Union of Conservation of Nature (IUCN) since 2008 with only a few hundred living in wild. However, little is known of the population structure of pygmy hog. Even the most fundamental aspects of population ecology, such as the effective population size, are unknown. Here, we analysed the whole genome sequencing data of six individual pygmy hogs: three from the wild and three from a captive population to access the current and past population history of pygmy hog. First, we show that pygmy hog has remained at small population sizes with low genetic diversity since ~1 Mya. Surprisingly, mitochondrial haplotype analysis indicated that the existing populations from both wild and captivity are coming from the same maternal lineage. Second, to determine the degree of inbreeding we searched the genomes for regions of homozygosity (ROHs). The ROH analysis suggests that pygmy hog has gone through past and not recent inbreeding which is in line with results from other small and inbred populations.

Effects of sex ratio bias on the evolution of parental roles

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Many studies show that sex differences in parental behaviour are related to biases in sex ratio. However, the causal relationship between parental roles and sex ratio bias is often unclear. Moreover, there is a considerable debate in the literature on whether the “adult sex ratio” (ASR, ratio of adult males to adult females) or the “operational sex ratio” (OSR, ratio sexually active males to sexually active females) is more relevant for the evolution of parental roles. Theoretical arguments are difficult to judge, since they tend to be based on the sophisticated (and error-prone) analysis of an abstract fitness function. In addition, they neglect the possibility of individual variation and the ability of organisms to make their parental behaviour dependent on their own state and on environmental conditions. Therefore, we studied the joint evolution of parental roles and various sex ratios in an individual-based simulation model. When we biased ASR (e.g. by sex-differential juvenile mortality), this had a strong effect on parental roles: uniparental female-care evolved when ASR was female-biased, while uniparental male-care evolved under a male-biased ASR. These patterns evolved irrespective of any bias in OSR. Quite commonly, an evolved parental care bias in favour of one sex was associated with an OSR bias in favour of the other sex. This happened, for example, when for males competition for mates was riskier than caring, they provided more care, while OSR was female-biased.

Teaching evolution using a card game: negative frequency-dependent selection

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Teaching biological evolution can be difficult for several reasons. Controversies surround the theory of evolution by natural selection, student confusion may arise because of preconceptions, and the material is simply complex. Furthermore, it has been suggested that active learning approaches may benefit student learning compared to traditional lecturing. For these reasons I designed a card game that can be used to teach evolution. Students work in groups of 4-6 to learn topics around the theme of protected polymorphism, or negative frequency-dependent selection. The active learning approach stimulates them to verbalize arguments and theories about selection, which may result in a deeper understanding than if they had only studied by reading and listening. Here I explain the game's learning goals and how the game is played.

Proximate causes of communal breeding in burying beetles: the importance of ecological and social factors on behavioural and reproductive strategies

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In social animals, individuals may cooperate to reproduce through communal breeding, if independent breeding is directly constrained by ecological factors. However, there is still no firm evidence to support ecological constraints and interspecific competition driving evolution of communal breeding in social animals. In burying beetles, where individuals use carcass to lay their eggs and raise their offspring, individuals always reproduce together on a large-sized carcass through communal breeding. Here, we investigated a suite of proximate causes affecting the evolution of communal breeding, and studied experimentally how both ecological factors (resource availability and intensity of competition) and intra-specific social interactions shape behavioural (i.e. dispersal behavior) and subsequent reproductive benefits, using the burying beetles, *Nicrophorus vespilloides*. We found that the occupation of large carcasses, but not the presence of inter-specific intruders significantly induced aggregation of individuals, but individuals did not increase their defense of large carcass against interspecific intruders. Reproductive success was similar for non-communally and communally breeding individuals, despite of larger broods being produced in communally breeding groups. Also, an individual's probability to stay with the carcass was influenced by its intrinsic characteristics (i.e. body size), not by extrinsic factors (i.e. carcass weight, number of breeders).

Evolutionary contingency drives metabolic interactions and the emergence of ecological structure in microbial communities

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Ecological interactions such as metabolite exchange are widespread in natural and experimental microbial communities, and are a key driver of ecosystem structure. Here we ask to what extent chance events in evolutionary history, given constant environmental conditions, determine the evolution of metabolite exchange.

To address this question we used Virtual Microbes (a general model to study microbial eco-evolutionary dynamics) to perform a parallel evolution experiment to evolve populations of digital microbes in a constant, one-niche environment.

Replicate evolutionary simulations, starting from the same population and only differing with respect to mutations, can result in communities where the complete metabolic network is partitioned over two stably co-existing lineages. Alternatively, communities of 'superorganisms' that can produce and recycle all essential metabolites can evolve. Which type of community evolves is contingent on prior metabolic adaptations. While the requirements for growth and reproduction are identical for all microbes, the large degrees of freedom offered by the Virtual Microbes model allows different metabolic solutions, and the evolved solution sets limitations on future ecological roles. This suggests that mutation-level events can blow up to shape whole ecosystems via new emergent functions.

Pteropods make thinner shells along a natural ocean acidification gradient

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Rising atmospheric carbon dioxide concentrations lead to ocean acidification (OA), which affects marine calcifying organisms. Shelled pteropods, a group of calcifying zooplankton, are widely regarded as bio-indicators of OA. Their delicate aragonitic shell can be damaged or even partially dissolve in waters with low aragonite saturation levels. However, pteropods can counter shell damage through extensive repair mechanisms under saturated conditions (Peck et al. 2018). Very little is known about natural variation in the total calcification of shelled pteropods. This work examines the impact of naturally occurring ocean acidification gradients on total calcification of *Limacina helicina* f. *pacifica* pteropods in the California Current Ecosystem. Here, we show with Micro-CT scans that shell thickness of *L. helicina* declines by 33.7% along a natural OA gradient generated by the combined effects of acidification and upwelling in the northern California Current Ecosystem. Low levels of genetic diversity ($\approx 0.26\%$) were found based on partial sequences of the mitochondrial COI gene, indicating a homogenous population. Shell thickness was decoupled from individual size, and thinner shells did not show more dissolution on the outer shell surface. Based on these results, we infer that pteropods make thinner shells, as they are affected in their net calcification by precipitating less shell material, rather than losing shell material, perhaps on account of an adaptive response

How organisms change their own selection pressure

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Ecosystem engineers modify their physical, abiotic environment, thereby facilitating other species. For instance, beavers constructing dams facilitate the occurrence of all kinds of fish and aquatic plants. In many cases the modifications also have profound effects for the engineers themselves: beavers experience lower predation pressure in the lake they created.

Ecosystem engineering may in fact be so widespread because it has been selected for. Yet, not much is known about the evolutionary component of ecosystem engineering. With the new and exciting field of eco-evolutionary dynamics, we are beginning to understand the complex relationship between ecology and evolution.

In this talk, I address what connects ecosystem engineering and evolution, what studies have been conducted in this regard, and what should be done to further integrate ecology and evolution. I will highlight our study system, where we investigate to which extent burrowing by the amphipod *Orchestia gammarellus* changes selection pressure of environmental stress, and the consequences of adaptation on salt marsh ecosystems.

The locus of eco-evolutionary dynamics: genetic and epigenetic determinants of life history in wild butterfly populations

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Some species evolve remarkably rapidly in response to abrupt changes in the environment, but this process is poorly understood. In particular, we crucially lack understanding of how genetic, evolutionary and demographic processes interact to determine adaptive capacity of populations. In order to identify limits and potential for rapid adaptation, 3 key questions need to be addressed. 1) What are the genetic and epigenetic mechanisms that regulate specific fitness traits in wild populations? 2) How is genetic variation for these mechanisms partitioned across populations and across the genome, and how does this relate to the distribution of neutral genetic variation? 3) What ecological, evolutionary and demographic processes maintain genetic variation for fitness traits in the wild? Here, we addressed these questions exploiting a classic model in eco-evolutionary dynamics, the Glanville fritillary butterfly. We studied two important fitness traits, lifespan and stress response, and combined these with detailed population genomic, transcriptomic, and epigenetic analyses. First, we identified genes associated with lifespan across three replicate populations, including hormone and neuronal signalling genes. Second, in lab experiments we identified stress-induced genes, including strong transgenerational effects. Finally, we studied variation, footprints of selection, and allelic turnover in space and time in these life history genes in the metapopulation.

The ancestor of *Termitomyces* was prone to be domesticated

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The old-world fungus-growing termites cultivate fungi of the basidiomycete genus *Termitomyces*. The symbiotic fungi descend from a single domestication some 30 million years ago. The termites propagate their fungi via asexual spores inoculated in their faeces. It has been hypothesised that asexual-spore production evolved after domestication as an adaptation to the symbiotic lifestyle. We test this hypothesis by reconstructing the evolution of asexual spores and coprophilia on a phylogeny based on whole-genome sequence data, including two recently described genera closely related to *Termitomyces*. We show that the non-domesticated genus *Arthromyces*, which is coprophilic and produces asexual spores, is the sister group of *Termitomyces*. Also in more distantly-related species, *coprophilia* and asexual spores are generally correlated, presumably because asexual spores allow the fast colonization of a temporary substrate. We conclude that two important traits of the domesticated fungus, viz. *coprophilia* and asexual-spore production, were already present in the ancestor of *Termitomyces*.

The role of recombination in the evolution of an antibiotic resistance enzyme

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The origin and evolution of recombination present a major problem in biology. Genetic recombination of closely related DNA sequences is fundamentally a random process that creates but also breaks up beneficial allele combinations. Experimental studies of recombination effects are not straightforward, because benefits may occur only over many generations and key variables are difficult to manipulate in experiments with living organisms. We use a radically different approach: the laboratory directed evolution of an antibiotic resistance enzyme. Directed evolution experiments combine in vitro mutation and recombination protocols with in vitro or in vivo selection and allow extreme control over evolutionary conditions and parameters. The enzyme we use - TEM β -lactamase - catalyzes the degradation of certain antibiotics, causing resistance of its bacterial host. By using random mutagenesis and selection of bacteria expressing different β -lactamase alleles in the presence of a novel antibiotic, we follow the step-wise evolution of the enzyme towards increased specificity for the new antibiotic. By introducing in vitro recombination at various rates and time intervals, we systematically explore conditions and time scales for recombination benefits and test the causes of the observed recombination benefits. Our ability to perform highly controlled evolution experiments allows rigorous tests of the evolutionary effect of recombination on a complex fitness landscape.

Wandering shells: the evolution of pteropod biomineralization

Paula Ramos-Silva, Frédéric Marin, Ferdinand Marlétaz and Katja Peijnenburg
Naturalis Biodiversity Center

Pteropods of the suborder Euthecosomata are a group of holoplanktonic gastropods that build thin and delicate shells of aragonite, a polymorph of calcium carbonate. Shelled pteropods have been proposed as biological indicators of ocean acidification (OA) due to their vulnerability to high CO₂ levels, including negative impacts on shell growth and dissolution. Still, these conclusions have been based on short-term experiments. The molecular basis of pteropod shell formation and its adaptive capacity remain overlooked. Similarly to other mollusks, pteropods build their shells through a biomineralization process controlled at the molecular level. The mantle is the primary organ: it expresses biomineralization genes and secretes shell matrix proteins (SMPs) that control shell formation and remain enclosed in the biomineral. By combining transcriptome data with shell proteomics, we directly identify SMPs from different pteropods, with coiled and uncoiled shells, and having distinct microstructures such as prismatic, crossed lamellar and helical fibers. A comparative analysis between the different sets of SMPs and shell microstructures elucidates the mechanisms of shell formation. Next, by mapping the presence/absence of shell-building genes in a time-calibrated pteropod phylogeny we contextualize the evolution of biomineralization in geological time and the expected responses to past, present and future climate changes.

Evolution and Development of Pollination Related Floral Traits in Phalaenopsis

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Naturalis Biodiversity Center

The variation in shape and size of different floral organs of orchid flowers is related to differently sized pollinators. In terms of evolution and development, the homology of these floral organs is complex as it involves duplication and modification of different developmental genes such as MADS-box genes. We performed micromorphological analysis (SEM and micro 3d-CT scanning) and transcriptome analysis to understand the evolution and development of callus, stelidia and gynostemium foot. We collected early stage and mature tissue samples from flowers of *Phalaenopsis equestris* (PE) and *Phalaenopsis pulcherrima* (PP), two species that differ significantly in terms of floral morphology: PE has a large callus but short stelidia and gynostemium foot, whereas PP has a small callus but long stelidia and pronounced gynostemium foot. Results of the SEM analysis show that the stelidia develops quite early, whereas the callus and gynostemium-foot develop much later. The micro 3D-CT scans show that the callus and stelidia are derived from staminodia, whereas the column foot has a mixed sepaloid-petaloid-staminodal origin. We currently investigate whether differences in floral morphology between the two species of *Phalaenopsis* are correlated with differential expression of developmental genes. If this is indeed the case, this could provide a genetic base for attracting differently sized bee species as pollinator.

Should I stay or should I go? – Consistent individual differences in migration tendency.

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Polymorphisms in life-history strategies and behavioural tendencies are wide-spread in nature and an outstanding question is how they are shaped by natural selection. Partial migration is an intriguing example. In many species, only a subset of individuals undertakes migration while the rest stays back in their natal environment. Studies have revealed that migrants tend to be a non-random subset of the population, differing in morphology (e.g., wings, armament), physiology (e.g., metabolic rate) and behavioural tendencies (e.g. boldness, exploration). We study partial migration in three-spined sticklebacks (*Gasterosteus aculeatus*), in river-based and land-locked populations in the North of the Netherlands. Their distribution over a variety of habitats, the occurrence of morphological and behavioural variation, their social and parental system, and the many tools available for their study make them ideally suited for investigating the causes and consequences of partial migration. Our first empirical results, based on the comparison of migrants with individuals from land-locked populations, show that there are systematic morphological and behavioural differences between the migratory and non-migratory forms, suggestive for the existence of a migration syndrome. We will discuss these differences in the light of various competing hypotheses and investigate the plausibility and empirical relevance of these hypotheses on the basis of individual-based evolutionary simulations.

The evolution of tooth replacement in deep-time

Martin Rücklin

Naturalis Biodiversity Center

The evolution of teeth and their replacement is mainly understood from extant conditions based on chondrichthyans, the most stem-ward crown gnathostomes. Their skeleton of minute dermal scales and cartilage fulfills expectations of the ancestral jawed vertebrate condition. Including the fossil record and phylogenetic congruence a secondary simplification leading to this condition is obvious. Fossil stem-chondrichthyans and stem-gnathostomes are needed to elucidate on character polarization and ancestral conditions.

Using digital non-destructive tomographic methods including synchrotron X-ray tomographic microscopy (SRXTM) sclerochronology and the pattern of tooth addition and replacement in fossil and extant forms is reconstructed.

Applying this approach to placoderms, a grade leading to crown-gnathostomes, the evolution of first teeth is evident. Teeth are added, but not shed arranged in rows. Stem chondrichthyans show a similar pattern of tooth addition, but possess also symphyseal tooth whorls of replaced teeth that are not shed. Files of shed and replaced teeth are a specialization of chondrichthyans. Stem osteichthyans and sarcopterygians have teeth that are resorbed before shedding. Tooth whorls might be a condition of crown gnathostomes, but are lost in tetrapods and persist in chondrichthyans arguing for a convergent evolution of tooth replacement mechanisms in chondrichthyans and tetrapods.

Does ontogeny matter? A simulation study on the effect of island ontogeny in the evolution of island clades

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Oceanic islands are not static entities; instead, their size and features change dynamically through time, a process called island ontogeny. It has been hypothesized that the gradual or at times abrupt changes an island experiences during its lifespan can have a strong effect on the composition and evolution of biological communities. The general dynamic model of island biogeography assumes that island area and topographic complexity change through time and influence the number of available niches, and thus rates of diversification. A previous study demonstrated the role of single-island ontogeny on the Hawaiian biota, but most diversification studies on islands have not considered it. We tested under what conditions island ontogeny can bias diversification studies if not taken into account, using a simulation approach based on an extended version of the phylogenetic framework DAISIE. We simulated evolution on islands subject to ontogenetic processes and attempted to infer the generating parameters using existing methods that ignore ontogeny. Furthermore, we assessed how reasonable predictions of future biodiversity that ignore past island ontogeny are. Our analyses allow us to lay out guidelines on how and when to incorporate island geomorphological data into island phylogenetic diversification studies. This will lead to more robust conclusions about the origin and maintenance of biodiversity on oceanic islands.

Epigenetics of animal personality: DNA methylation and its influence on exploratory behaviour in great tits (*Parus major*)

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Early developmental effects and environmental conditions experienced by parents affect personality traits, even over multiple generations. Yet, the mechanisms underlying transgenerational regulation remain unknown, while determining them is crucial to understand how development affects heritable traits in evolutionary processes. A likely mechanism involved in such epigenetic regulation is DNA methylation, since this can stably alter gene expression in response to environmental factors without structural modifications of the DNA sequence. We study this by associating DNA methylation to variation in exploratory behaviour in the great tit (*Parus major*). In order to do this, we manipulated brood size in a natural study population. We found more differentially methylated sites in the treatment set (enlarged vs. reduced brood size) than in the control set (control1 versus control2). Furthermore, we found differences in methylation percentage for genes related to growth and behaviour. Therefore, early life stress directly affects epigenetic factors related to growth and behaviour. In the future, we will assess if there is an effect of the observed methylation changes on variation in personality traits, if there is a genetic basis for (personality-related) DNA methylation and if gene methylation variation associates with behaviour and life-history traits under natural conditions.

The genetic basis of enhanced virulence in natural populations of an insect virus

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The *Spodoptera litura* nucleopolyhedrovirus (SpltNPV) infects the larvae of *S. litura* (leafworm) throughout the Asian continent, and potentially could be used for biological control of this pest insect. Differences in the biological activity of 22 SpltNPV isolates from Pakistan were found. The isolate SpltNPV-Pak-TAX1 kills *S. litura* significantly faster than SpltNPV-Pak-BNG. Fast action is considered essential for effective biological control with insect viruses. The full viral genomes of these two isolates were investigated by deep sequencing.

The consensus sequences of the two virus isolates share 99% identity, suggesting high relatedness. Major differences were observed in the number of homologous regions (HRs) – variable numbers of inverted repeated sequences – and the absence of ORF 125 in SpltNPV-Pak-BNG.

HRs potentially function as putative enhancer of transcription and origin of replication. The function of ORF125 is unknown. The rates of nonsynonymous and synonymous single-nucleotide substitutions (dN/dS) showed that overall purifying selection is acting on the genome. By contrast, ORF122 is probably under positive selection. This gene encodes a putative viral fibroblast growth factor-like protein (FGF-like), is involved in the passage of virus from the midgut to the interior of the larva, and is linked to virulence in other baculoviruses. Little polymorphism was observed within the two virus isolates. This result is at odds with observations for other baculoviruses,

We identified two loci that are possibly linked to the enhanced virulence of SpltNPV-Pak-TAX1. This information improves the understanding of the enhanced activity of SpltNPV-Pak-TAX1, and aids the selection of better SpltNPV isolates for the biocontrol purposes in Pakistan and elsewhere.

Characterization of controllable mutator strains

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The interplay between environmental selection and available variation within a population are central in the adaptation of populations to changing environments, for seemingly all levels of complexity in life. In order to investigate properties and mode of actions of the two counterplayers, more and more studies have started to conduct experiments, that expose populations to different, non-optimal conditions and describe changes in phenotypic traits and genotypes observed. Whereas the extrinsic selection regimes surrounding the populations can easily be changed at will by the investigators and hereby serves the elucidation of the properties of selection, modifying the intrinsic genetic variation of the individuals remains challenging and less of an experimental subject so far. In order to investigate mechanisms that cause genetic mutations, we constructed bacterial strains, that harbour mutator genes on plasmids with an inducible expression system. We present results of the characterization of the mutator strains, as a first step towards understanding mutation mechanisms and their influence on standing genetic variation and subsequent adaptation, in order to complement the investigations on selection for a full picture of the driving forces in evolution.

Evolution of antibiotic resistance in polymicrobial infections

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RUG

Communities of bacteria derived from polymicrobial urinary tract infections (UTIs) can be viewed as small ecosystems. By measuring the pair-wise interactions we obtained a unique insight in the ecological interactions of the UTI microbiome members. Based on these measured data we can elucidate the statistical network properties of these ecosystems, as well as the ecological stability of these communities.

Additionally, we discovered that many of these bacterial interactions affect the immediate tolerance to antibiotics, as well as the ability to evolve antibiotic resistance.

This may have consequences for the treatment and eradication of polymicrobial infections.

Highest plasticity of carbon concentrating mechanisms in earliest evolved phytoplankton

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Photosynthesis evolved in oceans of a distant past, when CO₂ partial pressure was high. Over time, pCO₂ dropped and primary producers required carbon-concentrating mechanisms (CCMs) to supply their carbon fixing enzymes with sufficient inorganic carbon (C_i). At present-day pCO₂, phytoplankton groups with an earlier origin exhibit a higher CCM activity with stronger intracellular C_i accumulation than their younger counterparts. Here, we tested whether earlier evolved phytoplankton groups also exhibit a higher CCM plasticity. To this end, we collected data from literature and applied a Bayesian linear meta-analytic model. Our results show that with elevated pCO₂, photosynthetic CO₂ affinities decreased strongest and most consistent for the earlier evolved groups, i.e. cyanobacteria and dinoflagellates, while CO₂-dependent changes in affinities for haptophytes and diatoms were smaller and less consistent. In addition, responses of maximum photosynthetic rates toward elevated pCO₂ were generally small and inconsistent across species. Our results demonstrate that phytoplankton groups with an earlier origin possess a high CCM plasticity while more recently evolved groups do not, which likely resulted from evolved differences in the CO₂ specificity of RubisCO.

Visual plasticity - a driver of cichlid divergence?

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Prior views have been dominated by the idea that phenotypic plasticity might constrain the stunning diversity of species. However, recent theories assume that plasticity plays a critical role in the early stages of speciation and adaptive evolution. It allows organisms to rapidly alter their phenotype according to environmental variations. Through facilitating niche expansion and exposing organisms to different selective pressures, it might drive species divergence. To adequately assess environmental cues, organisms require reliable sensory systems. This study will focus on visual perception, a crucial determinant of fitness in many taxa, it is mediated by photoreceptors, detecting different wavelengths of light depending on the absorption properties. Visual pigments are composed of an opsin protein covalently bound to a light-absorbing Vitamin-A-derived chromophore (i.e. either A1 or A2 derived). There are five major classes among opsin genes: rod opsin (Rh1), UV-sensitive (SWS1), Blue-sensitive (SWS2), Green-sensitive (RH2) and Red-sensitive (LWS). In this study, we investigate visual adaptation in cichlid fish, which form one of the most species rich families of vertebrates (i.e. 250-800 cichlids on each Lake). We aim to understand whether visual plasticity is a driver of cichlid divergence, through combination of behavioral trials (i.e. habitat choice) molecular data (i.e. opsin genotyping and chromophore expression) and phylogenetic comparative analysis.

Evolution in serial batch cultures: Selection pressures and conditions for coexistence

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Studying evolution in the laboratory has led to insights into evolutionary question, such as repeatability and contingency. For practical purposes, such experiments are often performed with microbes grown in liquid cultures which are propagated after a stationary phase is reached. Although practical, conceptually this environment is complicated—with a lag-phase, exponential growth phase, nutrient-limited growth phase and stationary phase—and the consequences for evolutionary adaptation are not yet fully understood. As the microbes evolve, they also change the dynamics of these different growth phases, allowing for an eco-evolutionary feedback and enabling the possibility of frequency dependent co-existence.

We derive the selection coefficient for the exponential and nutrient-limited growth phase in a liquid culture population in terms of maximal growth rate and substrate affinity. We analytically verify previous simulation results and we predict how the optimal strategy and the conditions for co-existence of a high growth-rate (r-strategy) strain with a high affinity (K-strategy) strain grown on a single resource depend on the external conditions, such as initial resource concentration.

We test these predictions qualitatively with in silico evolution of the Virtual Microbe a non-supervised model (i.e. it targets to combine relevant biological structures without a preconceived notion of "fitness", which is instead an emergent phenomenon).

Geographic variation in opsin expression does not align with opsin genotype in Lake Victoria cichlid populations

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Sensory adaptation to the local environment can be a potent force in speciation. Aquatic environments are well-suited for studying this: the natural attenuation of light through water results in distinct light environments, to which vision-dependent species must adapt. In the *Pundamilia* cichlid genus, sympatric species with blue vs. red male nuptial coloration co-occur at rocky islands in southern Lake Victoria. The species tend to be depth-differentiated, entailing different visual habitats. Divergent visual adaptation to these environments has been implicated as a major factor in the divergence of *P. pundamilia* and *P. nyererei*, that show consistent sequence differentiation in the long-wavelength-sensitive opsin (LWS). We characterized opsin expression and LWS genotype across populations, to examine how different mechanisms of visual tuning contribute to visual adaptation. As predicted, the short-wavelength-sensitive opsin (SWS2b) was expressed exclusively in populations from clear water. Contrary to prediction, expression of the other opsins were species- and island-dependent and did not align with species differences in LWS genotype. Specifically: in turbid water, the shallow-water dwelling blue species expressed more LWS and less RH2A than the deeper-dwelling red species, while the opposite pattern occurred at locations with clear water. Visual modeling suggests that the distribution of opsin expression profiles and LWS genotypes does not maximize visual performance.

Local continuous genetic Isolation-by-Environmental variation in the threespine stickleback (*Gasterosteus aculeatus*) in the Baltic Sea

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Evolutionary divergence across heterogeneous landscapes occurs even in the face of gene flow. The genetic underpinnings and scale at which this differentiation can occur in marine habitats is not well understood. In the Baltic Sea, threespine stickleback exist across large environmental gradients with no obvious barriers to gene flow. Despite this, previous studies found 5 genetic clusters based on 20 selected microsatellite loci linked to genes involved in local adaptation to environmental conditions. We tested if selection also occurs at a local scale on these stickleback, and how selected variation is related to morphology and environmental factors. We assessed genetic structure at these loci for 578 fish along a 400km latitudinal transect within one of the previously described clusters. At this scale, we detected significant isolation by distance (IBD, Mantel-test, $r^2 = 0.18$, $p < 0.01$) indicating that the selected genetic variation is not randomly distributed even within this cluster. Further analyses revealed that environmental rather than geographic distance is the main contributor to this IBD, indicating that environmental selection occurs at this scale. Our results revealed how local environmental variation shape genetic variation. This suggests that the genetic structure previously reported may reflect a continuous gradient rather than clusters and that stickleback can adapt to local environmental conditions despite continuous gene-flow in the rest of its genome.

Implications of adult sex ratio and dimorphism on courtship and fighting behaviour in the dung beetle *Onthophagus taurus*

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Adult sex ratio (ASR) is the proportion of males in the adult population of a species. ASR is a central concept of population demography and a key factor of evolution under sexual selection. Dung beetle *Onthophagus taurus* is an insect with typical dimorphism. In which male individuals have either small or large horns. This difference of ornament can also influence the sex selection process of insects. Here, we analyzed the implications of adult sex ratio and dimorphism on fighting and courting behaviours by using the dung beetle *Onthophagus taurus*. The experiments performed allowed to observe the fighting and courting behaviours of the beetles with both large and small horned males present in three different adult sex ratios (33, 50 and 66%). This showed a significant difference among the male-biased populations, the equal ASR and female-biased populations for the fighting behaviour of the beetles ($p < 0,05$). The horn size shows a significant effect on fighting behaviour, but not on courting behaviour. A significant interaction was also found between horn size and ASR on fighting behaviour of the male beetles, indicating only large horned males change their strategy according to ASR and becoming more aggressive as the percentage of males increases while small horned beetles show about the same fighting behaviour in all ASR setups. This indicates the fighting behaviour is dependent on an interaction between ASR and horn size traits, while courtship behaviour depends only on AS

Phylogenetic, morphological and ecological divergence in *Brachionus calyciflorus*, a newly described hybridizing species complex

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Brachionus calyciflorus is undoubtedly the most studied freshwater monogonont rotifer taxon. Although it has been identified to be a cryptic species complex since more than a decade, its four hitherto known species (*B. calyciflorus*, *B. dorcas*, *B. elevatus* and *B. fernandoi*) have been (re)described only recently, based on a combination of a molecular phylogenetic and morphological analysis. We here present the results of comparative life table and population demographic experiments using genotypes isolated from multiple natural populations in The Netherlands. The life table experiment revealed substantial life history strategy differentiation among species. Traits such as investment in sexual reproduction, juvenile and embryonic development time were found to be species-specific and also translated into divergent population demography structures. In addition, we applied cross-mating experiments between the two sibling species *B. elevatus* and *B. calyciflorus* to test for reproductive isolation. The results of these experiments suggest both pre- and post-zygotic isolation although formation of hybrids seems still possible. The latter is consistent with the occasional observation of hybrid and introgressed genotypes in natural populations. Our results demonstrate that correct identification is essential for the study of ecological dynamics in communities with 'cryptic' species.

Cracking the code of mutual signalling

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Sexual communication is generally assumed to consist of one sex being the signaller and the other sex being the responder. Yet, mutual signalling and resulting mutual mate choice is likely far more common, and should be considered to understand how sexual communication systems evolve. In the noctuid moth *Heliothis virescens*, females emit a sex pheromone to attract conspecific males from a distance. During close-range interactions males emit a related pheromone via elaborate structures, so-called hairpencils. We are investigating the extent of both male and female choice and female and male competition. Specifically, we are assessing how female and male pheromone compositions correlate with reproductive success, and which factors affect female and male quality and choice. The main challenge is to quantify and measure close-range courtship behaviours and disentangle male competition from female choice. By rearing the moths under different feeding conditions, we found that the female sex pheromone deteriorates in poor condition, while the male pheromone remains constant. However, males reared in poor condition had a lower reproductive output. Behavioural analyses indicate that females do not actively choose but mostly reject specific males. We are currently assessing under which circumstances females reject different quality males, and whether the rejection is based on the male pheromone or on other properties.